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A Preliminary Study of Formulations of Transfersomes for Improved Transdermal Peptide Delivery

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Abstract
Peptide-containing dosage forms are often administered through invasive injection. However, due to their great ultra-mobility, transfersomes offer a promising alternative for non-invasive and non-allergenic transdermal peptide delivery. Therefore, this study aimed to conduct preliminary investigations into the formulations of transfersomes for transdermal peptide delivery. Transfersomes were prepared using the thin-layer hydration method with Tween® 80 as an edge activator and Phospholipon® 90G as vesicle formers. Four formulations: F1, F2, F3, and F4 were optimized with various ratios of the two components, including 90:10, 85:15, 80:20, and 75:25. Particle size, zeta potential, deformability index, polydispersity index (PDI), and vesicle morphology were used to assess transfersomes. As a result, the zeta potential was -37.2 ± 2.19, the deformability index was 1.78 ± 0.03 and the smallest vesicle size (147 ± 1.93), PDI value of 0.105 ± 0.01 and spherical were all found in the optimal formula, F3, with an 80:20 ratio. These results suggest significant potential for the further development of transfersomes using Tween® 80 and Phospholipon® 90G as effective transdermal peptide delivery systems.

Keywords: Phosphatidylcholine, Transfersomes, Transdermal, Tween® 80

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Studi Pendahuluan Formulasi Transfersom untuk Meningkatkan Penghantaran Peptida secara Transdermal

Abstrak
Bentuk sediaan yang mengandung peptida sering diberikan melalui injeksi yang bersifat invasif. Transfersome adalah salah satu vesikel yang dapat digunakan sebagai penghantaran peptida melalui kulit yang bersifat non-invasif dan non-alergi, karena memiliki sifat deformabilitas yang tinggi. Penelitian ini bertujuan untuk melakukan studi pendahuluan terhadap formulasi transfersome sebagai sistem penghantaran peptide secara transdermal. Transfersome dibuat dengan metode hidrasi lapis tipis menggunakan Phospholipon® 90G dan Tween® 80, masing-masing sebagai pembentuk vesikel dan penggerak tepi. Formula F1, F2, F3, dan F4 dioptimasi dengan berbagai rasio kedua komponen, antara lain 90:10, 85:15, 80:20, dan 75:25. Transfersom dievaluasi dengan menentukan ukuran partikel, indeks polidispersitas (IPD), potensial zeta, indeks deformabilitas, dan morfologi vesikel. Hasil penelitian menunjukkan bahwa formula optimum adalah F3 dengan perbandingan 80:20, mempunyai ukuran vesikel paling kecil (147 ± 1,93), IPD 0,105 ± 0,01, potensial zeta -37,2 ± 2,19, indeks deformabilitas sebesar 1,78 ± 0,03, dan berbentuk bulat. Studi ini menunjukkan bahwa transfersom yang terbentuk dengan menggunakan Phospholipon® 90G serta Tween® 80 memiliki potensi untuk diformulasikan lebih lanjut sebagai sistem penghantaran peptida secara transdermal

Kata Kunci: Fosfatidilkolin, Transfersome, Transdermal, Tween® 80

1. Introduction

Peptide-containing dosage forms are often administered subcutaneously, intravenously, or intramuscularly.[1] These peptide products include abaloparatide, insulin, oxytocin, and salmon calcitonin.[2,3] However, the invasive nature of the approach may decrease patient compliance.[4] To address this issue, a non-invasive dosage form has been explored.[5] Various studies have been conducted to find alternative routes such as pulmonary inhalation or transdermal routes.[6] Peptides may penetrate systemic circulation through the transdermal route without degrading in digestion.[4] however, penetrating the stratum corneum is difficult.[7]

A promising approach to overcome the barrier of difficulty experienced in penetrating the stratum corneum is the development of nanovesicle delivery systems.[8] Examples of nanovesicle include liposomes, transfersomes, ethosomes, as well as niosomes that have been widely developed for transdermal delivery systems.[9] Transfersomes are the most promising among them in terms of delivering medications through the skin because of their deformability and capacity to shield the intracellular drug.[10] The development of transfersomes as a peptide delivery system has been studied, including their use with recombinant human epidermal growth factor and insulin.[11,12]

For the optimal formulation of transfersomes, it is important to use high-quality components with an adequate proportion of phospholipids and surfactants.[13] Therefore, in this study, transfersomes formulation was carried out with several comparisons of Phospholipon® 90G and Tween® 80.

2. Materials and Methods

2.1. Equipments

In this study, the equipments used included particle size analyzer type dynamic light scattering (Malvern nanosizer and zetasizer, United Kingdom), transmission electron microscope (TECNAI™ G2 Spirit Twin, Amerika), rotary evaporator (Buchi R-100, Switzerland), mini extruder kit (Avanti Polar Lipid Inc., USA), pH meter (Eutech Instrument pH 510, Singapura).

2.2. Materials

The materials used included phosphatidylcholine (Phospholipon® 90G was a gift from Lipoid GmbH (Köln, Germany), Tween® 80 (Merck, Germany), Sodium hydroxide (Merck, Germany), potassium hydrogen phosphate (Merck, Germany), butylated hydroxytoluene (Merck, Germany), and ethanol (Merck, Germany).

2.2.1. Preparation of Transfersomes

The formulations for the thin-layer hydration approach, which produced transfersomes, are displayed in Table 1. Butylated hydroxytoluene, Tween® 80, and Phospholipon® 90G were dissolved in ethanol, stored in a round-bottomed flask, and evaporated using a rotary vacuum evaporator at 150 rpm, 175 ± 5 mbar and 40°C.

The mixture was placed in a thin layer, subjected to nitrogen gas for two minutes, and then chilled for twenty-four hours to ensure complete solvent evaporation and to compact the thin layer.[3] To facilitate easy peeling, glass beads were used to hydrate the thin layer with phosphate buffer at pH 6.4 and 37°C. The round-bottomed flask containing the thin layer was initially rotated at 50 rpm, followed by an increase to 250 rpm for 45 minutes. The resulting dispersion of transfersomes was then extruded through a polycarbonate membrane with a 200 nm pore diameter at 37°C for 12 cycles.[3]

2.2.2. Characterization of Transfersomes

An ideal formulation was found by characterizing transfersomes and taking into account the following factors: vesicle morphology, zeta potential,

Table 1. Transfersomes formulations[3]

Materials	Concentration (% b/v)			
	F1	F2	F3	F4
Comparison Phospholipon® 90G and Tween® 80	(90:10)	(85:15)	(80:20)	(75:25)
Phospolipon® 90G	4.50	4.25	4.00	3.75
Tween® 80	0.50	0.75	1.00	1.25
Butylated hydroxytoluene	0.10	0.10	0.10	0.10
Phosphate buffer pH 6.40	ad 100	ad 100	ad 100	ad 100

F1 = Formula 1; F2 = Formula 2; F3 = Formula 3; F4 = Formula 4

polydispersity index, particle size, and deformability index.[4]

2.3. Particle Size, PDI, Zeta Potential

Particle size analyzer-type dynamic light scattering (PSA DLS) was used to measure the particle size, PDI, and zeta potential.[4] Distilled water was used to dilute the transfersome dispersion 200 times.[4]

2.4. Deformability Index (D)

Using the extrusion process and a 100 nm polycarbonate membrane, the deformability index was calculated. A milliliter of dispersion of transfersomes was loaded into the extruder and then extruded for five minutes, transfersomes' particle size, which passed the membrane, was measured using PSA DLS.[4,9] The following formula was used to determine the deformability index:

$$D = J\left(\frac{r_v}{r_p}\right)^2$$

where rv is the size of vesicles that pass through the membrane (nm), rp is the membrane pore size (nm), and J is the volume of fluid extruded for five minutes (ml).

2.5. Vesicle Morphology

Transmission electron microscopy (TEM) was used to analyze the morphology of the vesicles while adhering to the negative staining protocol. A 400-mesh copper grid coated with plastic and carbon was placed, plastic side down, on a sheet of parafilm containing a 5 ml liquid sample. The grid was left to absorb the liquid for about ten minutes. Filter paper was used to remove the extra liquid. Uranyl acetate (0.5%), which was filtered for 10 to 30 seconds, was used to stain the samples. The grid was put in the sample petri dish with the sample side facing up after any excess liquid was wiped off with filter paper. The samples were then examined under a 30,000x TEM magnification.[2,8]

2.6. Statistical Analysis

The data were examined using a one-way analysis of variance and the mean ± SD of three replicates, utilizing SPSS version 29.0 software. Additionally, it was deemed statistically significant when $P < 0.05$.

3. Results

3.1. Preparation of Transfersomes

The dispersion of transfersomes was milky white before extrusion and became more transparent afterward.

3.2. Characteristics of Transfersomes

Particle size, deformability index, zeta potential, and PDI are shown in Table 2. Transfersomes' formulations were F1, F2, F3, and F4 ordered from largest to smallest size. Based on Zaverage, the size of F4 vesicle was the smallest, namely 135.60 ± 2.09 nm. PDI values less than 0.2 were found in all formulations. Zeta potential of F1, F2, and F3 was more negative than -30 mV, however, F4 was less than -30 mV. Due to their complete membrane penetration, all transfersome formulations were deformable. Figure 1 displays the particle size distribution for each formulation.

Figure 2 shows the morphologies of F1, F2, F3, and F4, with spherical transfersomes being the end result. In all four formulations, the sizes obtained were less than 200 nm. These results were consistent with the particle size obtained using the PSA DLS.

4. Discussion

The thin-layer hydration method is widely used due to its simplicity and ease of implementation.[10,11] This method ensures consistent average diameters for each batch.[12,13] In line with these findings, vesicles size decreased with increasing transmembrane pressure as well as total extrusion cycles.[14]
A key factor in reducing particle size is the concentration

Table 2. Particle size, PDI, zeta potential, and deformability index

Parameters	F1	F2	F3	F4
Z average (nm)	163.87 ± 5.69	156.40 ± 2.69	147.20 ± 1.93	135.60 ±2.09
Polydispersity index	0.063 ± 0.05	0.116 ± 0.04	0.105 ± 0.01	0.108 ± 0.08
Zeta Potential	-36.27 ± 2.15	-40.63 ± 1.74	-37.20 ± 2.19	-28.30 ± 0.55
Deformability index	2.17 ± 0.11	1.82 ± 0.05	1.78 ± 0.03	1.52 ± 0.02

F1 = Formula 1, F2 = Formula 2, F3 = Formula 3, F4 = Formula 4
All values were defined as mean ± SD (n=3)

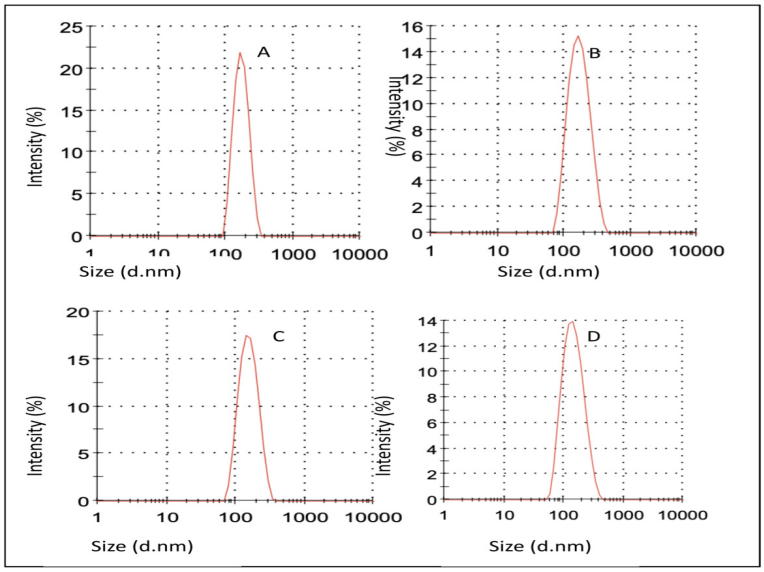

Figure 1. Transfersomes' formulation particle size distribution: A (F1), B (F2), C (F3), and D (F4)

ratio of Tween® 80 to Phospholipon® 90G. The extrusion results showed that higher surfactant concentrations led to a more transparent suspension, signifying smaller particle sizes.[17] The hydrophilicity of the Tween® 80 head contributed to vesicles size reduction.[6] However, the surfactant concentration should not be too high, as it can decrease the entrapment efficiency. This is due to an increase in the permeability of vesicle membranes caused by the arrangement of surfactant molecules within the lipid bilayer structure, resulting in the formation of pores within the membrane that can ultimately lead to leakage.[3] According to a previous study, the variation of Tween® 80 was 10 mg, 20 mg, and 30 mg, causing a reduction in size to 105,0 nm, 92,2 nm, and 85,0 nm, respectively.20 The same phenomenon was observed in this study, indicating that the larger the surfactant used, the smaller the size of vesicles obtained ($P < 0.05$). Particles up to 200 nm in size can pass through the transfollicular pathway.

PDI below 0.5, approaching 0, indicates a homogeneous sample.[18] The measurement of particle size distribution for values less than 0.5 was based on Zaverage which is calculated using the diameter

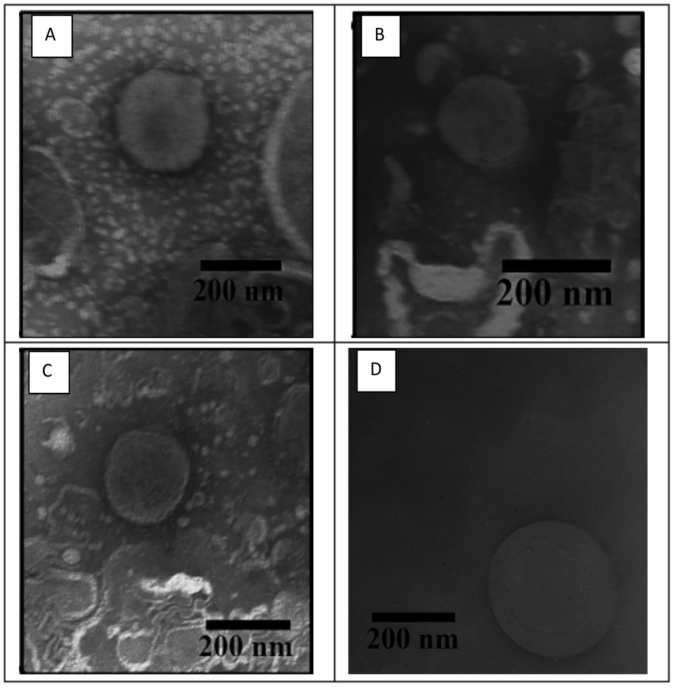

Figure 2. Transmission electron microscope image of transfersomes of A (F1), B (F2), C (F3), and D (F4) with a magnification of 30000x

of the light scattering intensity (dz).[19] Based on Zaverage, particle dimensions of the four formulations were below 200 nm due to the pore membrane used during the extrusion process. Increasing the amount of Tween® 80 and decreasing the amount of Phospholipon® 90G did not affect PDI value. PDI values below 0.2 (P > 0.05) for all formulations showed a good degree of particle size homogeneity. This is also because the extrusion cycle, carried out 12 times was sufficient to uniformly distribute particle size. According to a previous study, repeated extrusion can increase the homogeneity of the extruded vesicles.[21] A homogeneous vesicle population is indicated by a PDI of less than or equal to 0.3, which is deemed sufficient for drug delivery applications employing lipid-based vesicles like transfersomes.[18]

It is preferable for zeta potential values to be higher than +30 mV or lower than -30 mV.15 Zeta potential, a crucial indicator of the electrical potential or repulsion between particles in a suspended fluid, can have an impact on the dispersion method's strength. Moreover, this size shows flocculation or aggregation of particles in the dispersion.[15] The zeta potential is influenced by the pH of the dispersing medium. Phosphatidylcholine is a zwitterionic compound with an isoelectric point at pH 6. At its isoelectric point, phosphatidylcholine carries a net charge of zero, with positive and negative charges contributed respectively by the phosphate and amine groups. In this study, a phosphate buffer dispersing medium at pH 6.4 was used, which is slightly above the isoelectric point of phosphatidylcholine. Therefore, phosphatidylcholine was negatively charged under these conditions.[6] Zeta potential measurement results for the four formulations were statistically significantly different (P < 0.05). Zeta potential values are used to classify colloid stability and nanoparticle dispersions. These values include ± 0-10 mV (highly unstable), ± 10-20 mV (relative stable), ± 20-30 mV (moderately stable), and ± 30 mV (highly stable).[22]

Transfersomes, known for their high deformability, can transport active ingredients through lipid membrane hydrophilic pathways or cell gaps without compromising vesicles integrity. This is facilitated by surfactants acting effectively as edge activators.[16]

Among the formulations, F1 had the highest deformability index value, followed by F2, F3, and F4 (P < 0.05). In all four formulations, there was a decrease in vesicle size after extrusion due to the hydrophilicity of Tween® 80 head.[23] These show that transfersomes have elastic properties.
The reduction in surface free energy during the hydration of thin film lipids facilitates the formation of stable, closed vesicle structures with spherical and oval shapes, resulting in thermodynamic stability. After the extrusion process, vesicles remain intact because there is no damage.[24]

5. Conclusion

In conclusion, the thin-layer hydration approach was successfully used to manufacture transfersomes. Among the formulations tested, F3 was identified as the optimal formula, because it exhibited vesicle size (Zaverage) of 147.2 ± 1.93 nm, PDI of 0.105 ± 0.01, zeta potential of -37.2 ± 2.19 mV, good elasticity and deformability properties, and spherical morphology. These transfersomes' vesicles were suitable and had the potential to carry peptides.

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Conflict of Interest

The authors declare no conflicts of interest.

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