PHYSICAL STABILITY TESTING OF P-SYNEPHRINE PREPARED AS TRANSFERSOME GEL

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ABSTRACT

Objectives: While p-synephrine exhibits lipolytic activity, it also has a low oral bioavailability as well as hydrophilic characteristic, so it is difficult for it to penetrate the epidermis if it is made into transdermal preparation. The purpose of this research was to increase the penetration of p-synephrine by preparing it as transfersome gel.

Materials and Methods: Three transfersome formulas were prepared—F1, F2, and F3—with the surfactants used at Tween 80, Span 80, and the combination of Tween 80 and Span 80 with a ratio of 1:1, respectively.

Results: The results showed that F1 was the best formula, with the highest entrapment efficiency, of 64.058±0.754%, a particle size average of 103.3 nm, polydispersity index 0.269±0.05, and zeta potential of −36.2±0.64 mV, so this formula was employed for the gel formulation. Two gel formulas were then prepared, transfersome gel (GT) and non transfersome gel (GNT).

Conclusions: The two gels were evaluated for their physical stability, and GT was found to be more stable than GNT.

Keywords: Transfersome, p-synephrine, Transdermal, Penetration *in vitro* test.

INTRODUCTION

There is an increasing prevalence of overweight and obesity, which is considered a health problem. In 2014, the World Health Organization stated that more than 1.9 million adults were overweight (BMI ≥25 kg/m^2) [1]. An undesirable physical condition caused by overweight is the production and retention of cellulite, a result of fat deposits, typically in the thighs, buttocks, and hips, which create a dimpling, orange peel appearance on the skin [2]. Cellulite results from non-metabolized components including glucose, lipids, and proteins, causing a synthesis and accumulation of triglyceride in the adipocyte that leads to changes in the lipolysis mechanism. Lipolysis receptors are mostly found in the stomach, thighs, and buttocks, so massages and topical therapy in these places can reduce cellulite symptoms, by accelerating the lymphatic circulation and replacing the interstitial liquid [3].

A compound that reduces cellulite is p-synephrine, which has a lipolysis effect; it binds to β-3 adrenoceptors, increasing thermogenesis and lipolysis in adipose tissue [4]. Because p-synephrine is a hydrophilic compound, however, issues arise when it is used as an anti-cellulite gel in transdermal preparations. The lipid bilayer in the stratum corneum of the epidermis is hydrophobic, making it an obstacle to an active, hydrophilic compound, which it prevents from penetrating into the deepest layers of the skin [5].

The solution for this problem is to make the active ingredients into transfersome, a vesicle that can change the shape, elasticity, and flexibility and increase the entrapment of drugs and thereby facilitate their penetration into the skin tissue, enabling them to pass through the stratum corneum layer of the skin. Transfersome is ultradeformable (very easy to deform) because of the presence of surfactant, a hydrophilic and lipophilic group molecule that can combine a mixture of water and oil [6].

Surfactant is classified into three types cationic, nonionic, and amfoter [7]. In this study, we used a non-ionic surfactant because this has a low toxicity. Surfactants with different hydrophilic-lipophilic balance (HLB) values were used to investigate the different characteristics of the transfersome suspension produced, including their entrapment efficiency percentage. Tween 80 was used as a high HLB surfactant and Span 80 as a low HLB surfactant, with a third formulation combining a mixture of the two.

MATERIALS AND METHODS

Instruments

This study used following instruments: JEOL JEM 1400 transmission electron microscope (TEM) (JEOL, Pleasanton, California), Zetasizer Nano S particle size analyzer (Malvern Instruments, Malvern, England), Brookfield viscometer (Brookfield, Harlow, England), Shimadzu UV-1800 ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, Kyoto, Japan), mini extruder set (Avanti Polar Lipids, Alabaster, United States), Franz diffusion cell test equipment (Bengkel gelas, Jakarta, Indonesia), centrifuge (Sigma Laborzentrifugen, Osterode arm Harz, Germany), Hahn Shin HS-2005S-N rotary evaporator (Hahn Shin, Bucheon, South, Korea), vacuum evaporator (Janke and Kunkel IKA, Staufen, Germany), and ultrasonicator and homogenizer (Edmund Bühler, Hechingen, Germany).

Materials

The materials used were p-synephrine (Green Spring Technology, Shanghai, China), phosphatidylcholine (Lipoid, Steinhausen, Germany), dichloromethane (Merck, Darmstadt, Germany), methanol (Merck, Darmstadt, Germany), Tween 80 (Croda, Seraya Ave, Singapore), Span 80 (Croda, Seraya Ave, Singapore), carbomer (Lubrizol, Seoul, Korea), triethanolamine (TEA) (Petronas, Kuala Lumpur, Malaysia), sodium hydroxide (Brataco, Jakarta, Indonesia), potassium dihydrogen phosphate (Brataco, Jakarta, Indonesia), and aqua demineralisata (Brataco, Jakarta, Indonesia).

Methods

The transfersome was made using the thin layer method [8] with the formulation as shown in Table 1, with phospholipids and surfactant used at the ratio of 85:15. Formulations of Span 80 mixed with phospholipids were dissolved in dichloromethane and the mixture rotated by rotary evaporator at 50-150 rpm in a vacuum. When a thin layer formed, nitrogen gas was passed across it, and then it was placed in a refrigerator for 24 hrs. After that, the thin layer was hydrated using demineralized water.

In the hydration process, the Tween 80 formulation was dissolved in demineralized water with p-synephrine. This was rotated at 50-150 rpm using glass beads, not in a vacuum. The suspension formed was placed in a refrigerator for about 24 hrs, and then the particle size in the suspension was reduced by ultrasonication.

Transfersome characteristics

Morphology characteristics

A drop of transfersome suspension was dissolved in 50 drops of pH 7.4 phosphate buffer and then dropped on a grid and left until dry. Then, the vesicle's morphology was observed using a TEM.

Particle size and zeta potential characteristics

Particle size and zeta potential characteristics were ascertained using a particle size analyzer with dynamic light scattering method (Malvern Zetasizer) [3].

Entrapment efficiency

A 2-3 ml suspension was made using a centrifugation tube with filter spun at 4500 rpm for 48 hrs. Methanol was added to the suspension residue at the top of the tube, and the suspension was centrifuged again. Methanol was added to the resulting solution at the bottom of the tube (entrapped medicine concentration), which was then mixed using a vortex. After that, a mixture of phosphate buffer: ethanol (1:1) was added and mixed, again using a vortex (for total medicine concentration). Then, the absorption of both concentrations was measured using a spectrophotometer UV-Vis. The entrapment efficiency percentage was calculated as the concentration of entrapped medicine divided by the total of medicine concentration multiplied by 100 to give a percentage.

Deformability index

The deformability index was calculated using:

$$D = J \left(\frac{rv}{rp} \right)^2$$

Where, D=Deformability index, J=Amount of suspension transfersome that passed through the membrane in 5 minutes (mL), rv=Size of transfersome particles that passed through the membrane (nm), and rp=Size of membrane pore (nm) [3].

Gel preparation

Carbomers were dissolved in aqua demineralisata until they expanded; next, the mixture was homogenized at 1500 rpm, propylene glycol was added, and the mixture homogenized again; then, TEA was added, and the mixture homogenized once more. Finally, the suspension was added to the gel, and the resulting mixture homogenized one more time, at 1500 rpm.

Gel evaluation

Organoleptic observation was made to evaluate the form, color, and smell of the final products. We also measured the potential of hydrogen (pH), to acquire the same value as that of (the range of) the skin, and we measured viscosity, to check gel rheology.

Stability test

Tests of stability were conducted, at a low temperature (4±2°C), room temperature (27±2°C), and high temperature (4±2°C) [8].

RESULTS AND DISCUSSION

Characterization of transfersome

Morphology characteristics

The transmission electron microscopy showed that the suspensions contained a spherical shape vesicle, with particles of around 100-200 nm. The vesicle was clearly visible on the outside as a wrapping layer, determined as a phospholipid bilayer comprising the single lamellar vesicles (Fig. 1).

Particle size and zeta potential

All three formulations showed results that afforded the desired characteristics (Table 2), which were a polydispersity index (PDI) value in the range of 0-0.6, a zeta potential value of more positive than +30 mV or more negative than −30 mV. The smallest particle size was observed in the suspensions with tween as a surfactant (F1).

Entrapment efficiency

Entrapment efficiency test results were obtained by the direct method. The average percentage of entrapment efficiency for the p-synephrine in F1, F2, and F3 was 64.05±0.75%, 46.05±0.24%, and 56.21±1.55%, respectively. The highest percentage of entrapment was F1, therefore, which used tween as a surfactant. Factors affecting entrapment efficiency included the alkyl chain contained in the surfactants [9]. The longer the alkyl chain of the surfactant in Span 80, the lower the capacity of entrapment will be. If the HLB value is 8.6 or more, the surfactant will have a high entrapment efficiency, while if the value of HLB is around 8.6 to 1.7, entrapment efficiency will decrease.

Gel evaluation and stability test

The gel evaluation results showed that the GT was white in color, while the GNT was transparent; both gels had an aroma similar to that of carbomers. The pH of GT and GNT was 5.48 and 5.70, respectively. The viscosity results at 20 rpm were 8920 cps for GT and 8200 cps for

Table 1: The formulation of p-synephrine transfersome

Material	Concentration % (b/b)		
	F1	**F2**	**F3**
Soybean Phosphatidylcholine	2.5	2.5	2.5
Tween 80	0.441	-	0.22
Span 80	-	0.441	0.22
P-synephrine	1	1	1
Demineralized water	Ad 100	Ad 100	Add 100

Table 2: Particle size, PDI, and zeta potential of p-synephrine transfersome

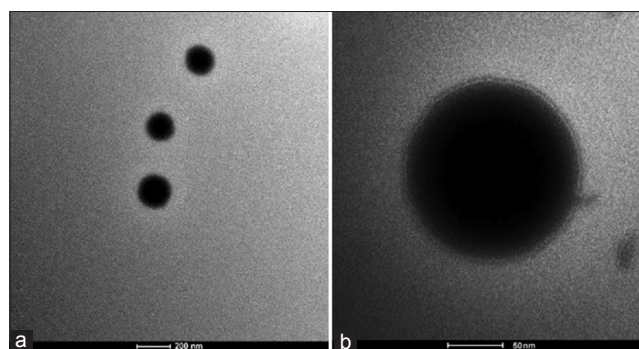| Sample	Particle size	PDI	Zeta potential (mV)
F1	101.93±0.55	0.264±0.011	−36.20±0.69
F2	171.00±14.06	0.358±0.009	−33.76±1.88
F3	110.03±3.82	0.248±0.006	−43.13±0.86

PDI: Polydispersity index

Fig. 1: Transmission electron microscopy results of p-synephrine transfersome suspension, (a) magnification of ×9900, 200 kV and (b) magnification of ×38,000, 200 kV

GNT. The transfersome gel had a higher viscosity because it contained phospholipid [5,10]. Both gels had a plastic thixotropic flow [11-13]. The results for organoleptic and homogeneity in the physical stability test of GT and GNT at low temperature (4±2°C), room temperature (25±2°C), and high temperature (40±2°C) were unchanged, as were the colors of GT and GNT and their smell during storage. The gels' pH changed, but they were both still at the pH range of the skin. In a test cycling, the gels did not change organoleptically, and syneresis did not occur.

CONCLUSION

From this study, it was concluded that F1 showed the best formula among three formulas, with the highest entrapment efficiency, a particle size average, PDI, and zeta potential. Therefore, this formula was employed for the two gel formulation (GT and GNT). The two gels were evaluated for their physical stability, and GT was found to be more stable than GNT.

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