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Optimization of Soya Phosphatidylcholine and Tween 80 as a Preparation of Diclofenac Sodium Transfersome Vesicles Using Design-Expert

(*Optimasi Fosfatidilkolin Kedelai dan Tween 80 Sebagai Penyusun Vesikel Transfersom Natrium Diklofenak Menggunakan Design-Expert*)

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ABSTRACT

Background: Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) widely prescribed for inflammation and pain. However, when used orally, diclofenac sodium has poor bioavailability because it undergoes *first-pass metabolism* in the liver, so only about 50% of the drug reaches systemic circulation. Therefore, the transdermal delivery system, in this case, transfersome nanovesicles, was chosen as an alternative to overcome this problem. Transfersome is a lipid vesicle with the best deformability in penetrating the skin layer among other nanovesicles. Transfersomes consist of active substances, phospholipids, surfactants, and other ingredients. The composition of phosphatidylcholine as a phospholipid and tween 80 as a surfactant is a variable that can affect the optimization of the transfersome formula. Therefore, the ratio of phospholipids and surfactants should be varied to obtain the most stable transfersome formula with high drug entrapment efficiency. **Objectives:** This study aims to determine the ratio of soya phosphatidylcholine as a phospholipid and tween 80 as a surfactant in the optimum formula of diclofenac sodium transfersome vesicles using *Design-Expert* and to determine the characteristics of the resulting transfersome vesicles. **Material and Methods:** Optimizing the transfersome Diclofenac Sodium formula using the *factorial design* 2^2 with soya phosphatidylcholine and tween 80 factors, particle size response, and entrapment efficiency. The thin layer hydration method carried out the process of making diclofenac sodium transfersome. **Results:** The results obtained from this study, namely the optimum formula based on *Design-Expert*, obtained a ratio of soya phosphatidylcholine and tween 80 of 4.5%: 0.5%. The results of the characterization of the optimum formula obtained a particle size of 224.3 nm, a zeta potential of -57.1 mV, and entrapment efficiency of 99.85%. **Conclusions:** The results of the characterization of the diclofenac sodium transfersome have met the specifications required for each test.

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ABSTRAK

Latar Belakang: Natrium diklofenak adalah salah satu obat antiinflamasi nonsteroid (NSAID) yang banyak diresepkan untuk inflamasi dan nyeri. Namun dalam penggunaannya secara oral, natrium diklofenak memiliki bioavailabilitas yang buruk karena mengalami *first pass metabolism* di hati sehingga hanya sekitar 50% saja obat yang sampai pada sirkulasi sistemik dan jika digunakan dalam jangka panjang maka akan menyebabkan efek samping terhadap gastrointestinal dan kardiovaskular. Oleh karena itu, sistem penghantaran transdermal dalam hal ini nanovesikel transfersom dipilih sebagai alternatif untuk mengatasi permasalahan-permasalahan tersebut. Transfersom adalah suatu vesikel lipid yang memiliki deformabilitas paling baik dalam menembus lapisan kulit diantara nanovesikel lainnya. Transfersom terdiri dari zat aktif, fosfolipid, surfaktan dan bahan lainnya. Bahan dan Metode: Penelitian ini bertujuan untuk mengetahui perbandingan fosfatidilkolin kedelai sebagai fosfolipid dan tween 80 sebagai surfaktan pada formula optimum vesikel transfersom natrium diklofenak menggunakan *Design-Expert* dan untuk mengetahui karakteristik vesikel transfersom yang dihasilkan tersebut. Optimasi formula transfersom natrium diklofenak menggunakan metode *factorial design* 2^2 dengan faktor fosfatidilkolin kedelai dan tween 80 serta respon ukuran partikel dan efisiensi penjerapan. Proses pembuatan transfersom natrium diklofenak dilakukan dengan metode hidrasi lapis tipis. Hasil: formula optimum berdasarkan *Design-Expert* diperoleh perbandingan fosfatidilkolin kedelai dan tween 80 sebesar 4.5% : 0.5%. Hasil karakterisasi formula optimum diperoleh ukuran partikel sebesar 224.3 nm, zeta potensial -57.1 mV dan efisiensi penjerapan senilai 99.85%. Kesimpulan: Hasil karakterisasi dari transfersom natrium diklofenak telah memenuhi spesifikasi yang dipersyaratkan dari masing-masing ujinya.

Kata kunci: Transfersom, natrium diklofenak, *Design-Expert*, karakteristik.

INTRODUCTION

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug (NSAID) that is widely prescribed for inflammation and pain. DS is widely used by the people of Indonesia as an oral drug (Noval and Rosyifa, 2021). In fact, the oral route of diclofenac sodium bypasses first-pass metabolism in the liver so that only about 50% of the drug reaches systemic circulation. This extensive metabolism is the reason why DS has poor oral bioavailability (Yuan et al., 2017). Oral administration of diclofenac sodium can also cause gastrointestinal and cardiovascular side effects (Dalimunthe and Ricky, 2021). Therefore, the transdermal delivery system was chosen as an alternative to overcome these problems. This is in accordance with Ernawati's research (2017) that the transdermal route can eliminate fluctuations in absorption in the gastrointestinal tract, can increase the bioavailability of drugs because the active ingredients will enter directly into the circulatory system through the skin and avoid the hepatic first-pass effect. In addition, the transdermal route can provide a constant and controlled drug input as well as reduce variations in plasma drug levels, improve patient compliance due to easier administration, and have minimal risk of injury, pain, or tissue damage.

The biggest obstacle in the delivery of the transdermal route is the presence of a layer of stratum corneum on the outermost skin, which is tightly packed so that it is difficult for molecules to penetrate from the outside. Various approaches have been taken to increase penetration through the stratum corneum, including using a nanovesicle carrier system. Nanovesicle is one of the delivery systems for transdermal preparations in the form of a bag with a nanometer size. Nanovesicles consist of liposomes, niosomes, and transfersomes. Transfersomes can deliver drugs with various solubility properties. Transfersome is

also elastic, so they can pass through gaps that are 5 to 10 times smaller without losing their shape. The elasticity and deformability of transfersomes are its advantages to more easily penetrate the skin layer so that drugs can more easily enter the systemic circulation (Ratnasari and Effionora, 2016). Transfersome is a lipid vesicle that has the best deformability among other nanovesicles (Ramadon and Abdul, 2016).

Transfersomes consist of phospholipids and edge activators (EA), which are substances that increase membrane flexibility and facilitate the ultra-deforming properties of transfersomes (Opatha et al., 2020). Transfersomes use phosphatidylcholine and surfactants as the two main components of their vesicle formation (Sugiyati et al., 2015). Soya phosphatidylcholine was chosen as a phospholipid in this study because it has a greater membrane deformability index than egg phosphatidylcholine, so the interaction ability of soya phosphatidylcholine with surfactants will be higher (Nurmahliati et al., 2020). At the same time, the surfactant/edge activator used in this study is tween 80. This is based on various studies which reported that transfersomes made using tween 80 have better flexibility and permeation and have smaller particle sizes with high drug entrapment efficiency, compared to other types of surfactants, such as sodium cholate, span 20, and span 80 (Sunday et al., 2017).

The composition of phosphatidylcholine as a phospholipid and tween 80 as a surfactant is a variable that can affect the optimization of the transfersome formula. Therefore, the ratio of phospholipids and surfactants should be varied to obtain the most stable transfersome formula with high drug entrapment efficiency (Nurmahliati et al., 2020; Sari et al., 2020; Dalimunthe and Ricky, 2021). Optimization as an approach to get the best combination of a formula can be done in a more efficient way using software called Design-Expert. This software is used to help carry out experimental designs, such as determining the optimum formula for a preparation (Hidayat et al., 2021).

Based on the explanation, a transfersome formula from Diclofenac Sodium was made, which included optimization of the ratio of soya phosphatidylcholine and tween 80 concentrations using design-expert. This research is expected to be the new innovation for transfersome nanovesicle preparations, especially for diclofenac sodium.

MATERIAL AND METHODS

Materials

The materials used in this study included diclofenac sodium (Fagron Hellas, Greece), ethanol 96% pro-analyst (Merck, Germany), soy phosphatidylcholine (Archer Daniels Midland Company, USA), tween 80 (Bratachem, Jakarta), Phosphate Buffer Saline (PBS) pH 7.4 (Merck, Germany), aluminum foil, parchment paper, 0.22 µm (Nylon) filter paper.

Methods

The formulation of transfersome vesicles in this study used the thin-layer hydration method. Diclofenac sodium transfersome vesicles were prepared using selected solutions, namely phospholipids, surfactants, ethanol, and phosphate buffer (Table 1). Determination of the composition of the formula is made by designing the composition of the formula between active substances, phospholipids, surfactants, ethanol, and phosphate buffer. 50 mL of diclofenac sodium transfersome vesicles were made with the following formula:

Table 1. Formulation of Transferosome

Material	Concentration	Function
Diclofenac Sodium	50 mg	Active ingredient
Soya phosphatidylcholine	4 – 4.5 %	Vesicle Forming
Tween 80	0.5 – 1 %	Surfactant/Edge Activator
Ethanol	10 %	Solvent
Phosphate Buffer Saline (PBS)	ad 100 %	Hydrating medium

The formula optimization was determined using Design-Expert software based on comparing the concentration of soy phosphatidylcholine and surfactant tween 80 with the desired criteria: entrapment efficiency (%EE) and particle size.

Table 2. Comparison of soya and tween 80 phosphatidylcholine concentrations using the Design-Expert factorial method 2^2.

Run	Factor 1 A : Soya Phosphatidylcholine (%)	Factor 2 B : Tween 80 (%)
1	4	0.5
2	4.5	1
3	4	0.5
4	4	1
5	4.5	0.5
6	4.5	1
7	4	1
8	4	1
9	4.5	1
10	4.5	0.5
11	4.5	0.5
12	4	0.5

The manufacture of transfersomes based on the ratio of the concentration of phosphatidylcholine soy and tween 80 using the Design-Expert factorial method was carried out with three replications to obtain the optimum transfersome formula. Transfersomes made with the optimum formula were then characterized to determine particle size, entrapment efficiency, and zeta potential.

Transfersome Vesicle Characterization

Determination of Vesicle Morphological Size

Transfersome suspension is placed on the surface of the slide. The shape and size of the vesicles were observed using an optical microscope with a magnification of 100 times (Dalimunthe and Ricky, 2021).

Furthermore, the particle morphology images from the microscope were analyzed for size using ImageJ software (Kurniawan et al., 2011).

Entrapment Efficiency (%EE)

The amount of adsorbed and non-absorbed drugs was measured by calculating the entrapment efficiency. The first step was centrifugation for 60 minutes at 6000 rpm for each diclofenac sodium transfersome colloid. The clear-colored supernatant was calculated for the drug content using a UV-VIS spectrophotometer as a result of centrifugation. The entrapment efficiency (EE) was expressed by the percentage difference between the amount of drug introduced into the transfersome system and the amount of free drug in the supernatant (Equation 1)

$$\%EE=(TD-FD)/TD \times 100\%$$

Equation 1. EE = Entrapment Efficiency; TD = Total compounds contained in the formula; FD = The number of compounds in the supernatant (not adsorbed) (Kuncahyo et al., 2021).

Characterization of Optimum Formula of Diclofenac Sodium Transfersome Vesicles

Entrapment Efficiency (%EE)

The entrapment efficiency (AE) was expressed by the percentage difference between the amount of drug in the transfersome system and the amount of free drug in the supernatant.

Particle Size

Measurement of transfersome particles was measured by dissolving 100 µL of the sample in 4 mL of phosphate buffer solution pH 7.4 and then using a Particle Size Analyzer (PSA) (Nurmahliati et al., 2020).

Zeta Potential

Zeta potential was measured using a zeta sizer, this is done by diluting transfersome suspension using distilled water. 10 ml of the diluted sample was slowly placed into the cuvette, then the cuvette is inserted into the tool, and the zeta potential is measured (Ratnasari and Effionora, 2016).

RESULTS AND DISCUSSION

Transfersomes as nanovesicle carriers were formulated with the active substance of 50 mg Diclofenac Sodium in 50 ml transfersomes and were intended for transdermal delivery. Transfersome vesicle-forming components consist of phospholipids, surfactants (edge activators), solvents, and hydrating media. The phospholipid used is soya phosphatidylcholine. Soya phosphatidylcholine was chosen because it has a greater membrane deformability index than egg phosphatidylcholine, so the interaction ability of soy phosphatidylcholine with surfactants will be higher (Nurmahliati et al., 2020). While the surfactant/edge activator used is tween 80. This is based on various studies which reported that

transfersomes made using tween 80 have better flexibility and permeation and have smaller particle sizes with high drug entrapment efficiency compared to other types of surfactants such as sodium cholate, span 20, and span 80 (Sunday et al., 2017). The solvent used is 96% ethanol, a flexible solvent that can dissolve the components that make transfersomes is not toxic compared to other organic solvents, is not easily overgrown by microbes, and is relatively inexpensive (Muthmainnah, 2017). The hydrating medium used is Phosphate Buffer Saline pH 7.4, whose use is intended to maintain the drug concentration and surfactant concentration (edge activator) (Dudhipala et al., 2020).

Diclofenac sodium transfersome was prepared using the thin layer hydration method. This technique is widely used because of its simplicity, practicality, and ability to produce small and uniform vesicles with high drug entrapment efficiency (Ahad et al., 2017). This technique is carried out by first dissolving a certain amount of soya and tween 80 phosphatidylcholine in ethanol, then adding the active substance diclofenac sodium to the mixture. Evaporated using a rotary vacuum evaporator at a temperature of 40^{o}C at a speed of 200 rpm to evaporate organic solvents. Then, it was allowed to stand for 24 hours in a desiccator to complete the formation of vesicles. The resulting thin layer was hydrated using Phosphate Buffer Saline (PBS) pH 7.4 and then stirred at a temperature of 56^{o}C at 75 rpm for 30 minutes. The suspension formed was then sonicated with an amplitude of 40 Hz for 30 minutes. The purpose of sonication is to obtain a smaller and more homogeneous particle size. The transfersomes obtained were then filtered with 0.22 m filter paper and transferred into a vial (Ambarwati and Yulianita, 2019).

Optimization of Transfersome Diclofenac Sodium Formula

The formula optimization was carried out using the Design-Expert software with three replication of the factorial 22 design. The independent variables were included along with each variable's upper and lower, namely soy phosphatidylcholine (4-4.5%) and tween 80 (0.5-1%). In addition, the desired response is also included, namely the entrapment efficiency (95-100%) and the particle size (70-400 nm). The results of running the formula using the Design-Expert factorial design method 2^2 resulted in 12 run formulas which can be seen in Table 2. Transfersome Diclofenac Sodium with a ratio of soy and tween 80 phosphatidylcholines, was then formulated for further characterization in order to obtain the response value on Design-Expert.

Characterization of Diclofenac Sodium Transfersome

Determination of Vesicle Morphological Size

The results of running 12 formulas on Design-Expert resulted in 4 main formula comparisons, namely the ratio of phosphatidylcholine and tween 80 of 4.5:1 (F1), 4.5:0.5 (F2), 4:1 (F3), and 4: 0.5 (F4). The results of morphological observations using an optical microscope with a magnification of 100x on the four formulas can be seen in Figure 1.

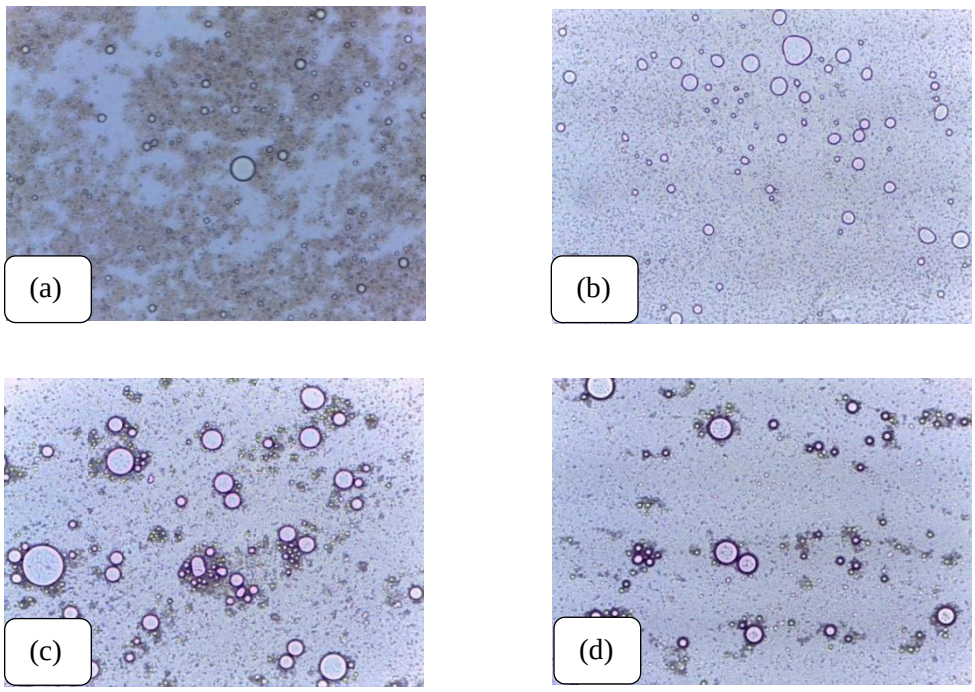

Figure 1. Morphology of diclofenac sodium transfersome vesicles with: (a) Formula 1 (4.5% of Soya Phosphatidylcholine: 1% of Tween 80); (b) Formula 2 (4.5% of Soya Phosphatidylcholine: 0.5% of Tween 80); (c) Formula 3 (4% of Soya Phosphatidylcholine: 1% of Tween 80); (d) Formula 4 (4% of Soya Phosphatidylcholine: 0.5% of Tween 80)

The image of each formula was then determined by the size of the vesicle using ImageJ software, with the results as shown in Table 4.

Entrapment Efficiency (%EE)

Before determining the value of entrapment efficiency, a standard curve of diclofenac sodium was made at concentrations of 10, 20, 30, 40, and 50 ppm. The purpose of making a standard curve for diclofenac sodium is to obtain a linear equation y = a + bx, which serves to calculate the total concentration of diclofenac sodium in the sample. The absorbance results as shown in Table 3 are then regressed to the concentration resulting in a line equation y = 0.011x + 0.254 with a linearity correlation value of r = 0.999, an intercept value a = 0.011 and a slope b value = 0.254.

Table 3. Absorbance values for variations in the concentration of Diclofenac Sodium

Concentration (ppm)	Absorbance
10	0.378
20	0.487
30	0.600
40	0.723
50	0.847

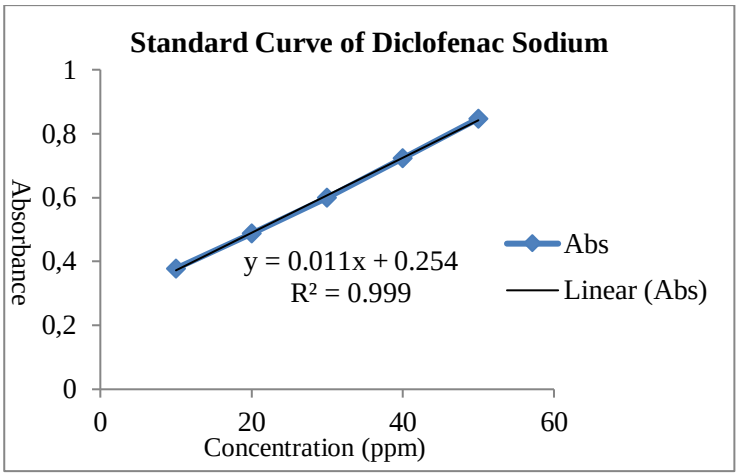

Figure 2. Graph of the Standard Curve of Diclofenac Sodium

The curve in Figure 2 shows a linear graph, which means that every increase in the concentration value is followed by an increase in the absorbance value. After knowing the values of x and y based on the standard curve of diclofenac sodium, the value of the entrapment efficiency can be calculated, and the results are obtained as shown in Table 4.

Optimum Formula for Transfersome Diclofenac Sodium

The optimum formula is assumed to be a formula that can meet the response criteria and the level range of each factor using the factorial design method. The response results obtained to determine the optimum area with the factorial design method can be seen in Table 4.

Table 4. Measurement results of particle size and entrapment efficiency responses

Run	Factor 1	Factor 2	Response 1	Response 2
	A: soya phosphatidylcholine (%)	B: Tween 80 (%)	Particle Size (nm)*	Entrapment efficiency (%)*
1	4	0.5	212.98±0.85	99.18±1.12
2	4.5	1	70.38±0.32	99.74±0.85
3	4	0.5	177.76±0.21	99.19±1.02
4	4	1	106.98±0.80	99.32±0.94
5	4.5	0.5	85.97±0.32	99.52±0.75
6	4.5	1	81.24±0.12	99.70±1.01
7	4	1	97.25±0.22	99.30±1.21
8	4	1	125.23±0.88	99.31±1.11
9	4.5	1	72.65±0.21	99.69±0.95
10	4.5	0.5	84.54±0.32	99.51±0.85
11	4.5	0.5	95.57±0.41	99.54±1.01
12	4	0.5	170.01±0.92	99.21±0.88

*Data is given in mean±SD in three replicates

The maximum desirability value determines the most optimum formula. Desirability is a function value that shows the program's ability to fulfill the wishes based on the criteria set in the final product. The desirability value that is getting closer to 1.0 indicates the program's ability to produce the desired product is improving (Ramadhani et al., 2017). In its application, Design-Expert issued seven selected

formulas. The formula with a desirability value closest to 1.0 is in the first, which is worth 0.964, as shown in Table 5. The results from Table 4 show that the optimum formula is in the desirability of 0.964. To see if there is an interaction that occurs is necessary to analyze the interaction graph and contour plot, that can be seen in Figure 3. Interaction graphs and contour plots will show a response that will change depending on the level of the specified factor.

Table 5. Selected Formula based on Desirability Value

No	Soya phosphatidylcholine (%)	Tween 80 (%)	Particle size (nm)	Entrapment efficiency (%)	*Desirability*	
1	**4.500**	**1.000**	**74.762**	**99.716**	**0.964**	***Selected***
2	4.500	0.981	75.302	99.709	0.963	
3	4.488	1.000	75.594	99.707	0.962	
4	4.500	0.966	75.723	99.703	0.961	
5	4.500	0.940	76.424	99.694	0.959	
6	4.474	1.000	76.620	99.695	0.959	
7	4.500	0.669	83.983	99.593	0.938	

Based on the contour plot graph in Figure 3, it can be obtained data that the higher the concentration of soya phosphatidylcholine and tween 80, the smaller the particle size obtained. Soya phosphatidylcholine concentration has a great influence on particle size. The results of this study are correlated with research conducted by Vasanth et al., (2020) where the transfersomes made showed a decrease in particle size when the amount of soya phosphatidylcholine increased.

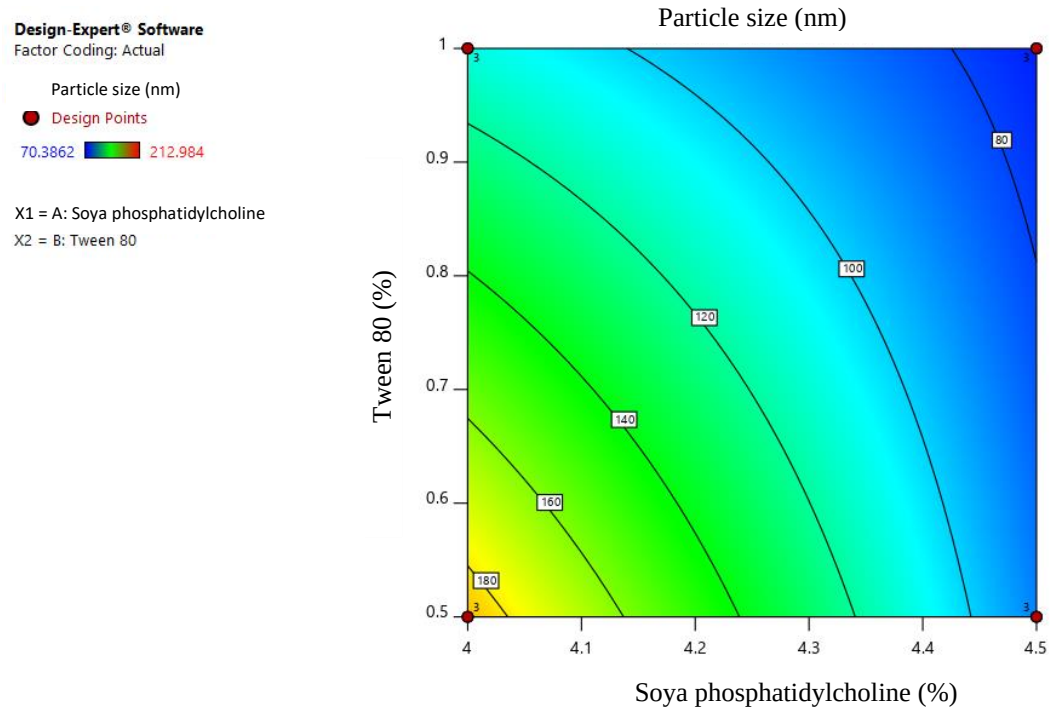

Figure 3. Particle Size Contour Plot Graph

The contour plot graph in Figure 3 also describes the interaction between the two factors on the particle size response. This is shown in dark blue to yellow on the chart. The yellow color on the graph shows a significant interaction at the concentration of tween 80. Where, the transfersome particle size gets bigger when the surfactant concentration is lowered and the transfersome particle size gets smaller when the surfactant concentration is increased. This is in accordance with research by Rahayu, 2019 which formulated mangosteen peel extract into transfersomes with various concentrations of tween 80 surfactant including 10%, 15% and 20%. The smallest transfersome particle size was produced by transfersomes with the highest surfactant concentration of 20%. Increasing the surfactant concentration will reduce the size of the particles. This happens because the increase in surfactant concentration can increase surfactant entrapment so that it can reduce the interfacial tension of the particles which causes the particles to be easily broken down and facilitate the formation of particles with smaller sizes (Elfiyani et al., 2017).

Based on the graph of the contour plot in Figure 4, it can be seen that increasing the concentration of soya phosphatidylcholine and tween 80 can increase the entrapment efficiency. On the graph there is an arc that points to the top right of the graph. This shows that there is an interaction between the two factors that both increase or decrease the response of entrapment efficiency when the concentration changes. These results are in line with the study conducted by Vasanth et al., (2020) who reported that for hydrophobic drugs, there was an interaction between the phospholipids used and the edge activator/surfactant that caused a change in the entrapment efficiency (%EE). Increasing the concentration of soya phosphatidylcholine can help increase %EE as well as edge activator. The surfactant concentration affects %EE because when used at low concentrations, the monomers that make up the surfactant can combine themselves through the lipid bilayer and this will limit the growth of vesicles, while at increasing surfactant concentration, the vesicles will grow and increase the fluidity of the membrane leading to an increase in the drug content trapped. This is advantageous in the formulation of hydrophobic compounds because drug molecules can be dispersed and trapped in the lipid matrix. This study showed a decrease in the sodium entrapmnet efficiency of diclofenac along with a decrease in soya phosphatidylcholine and tween 80.

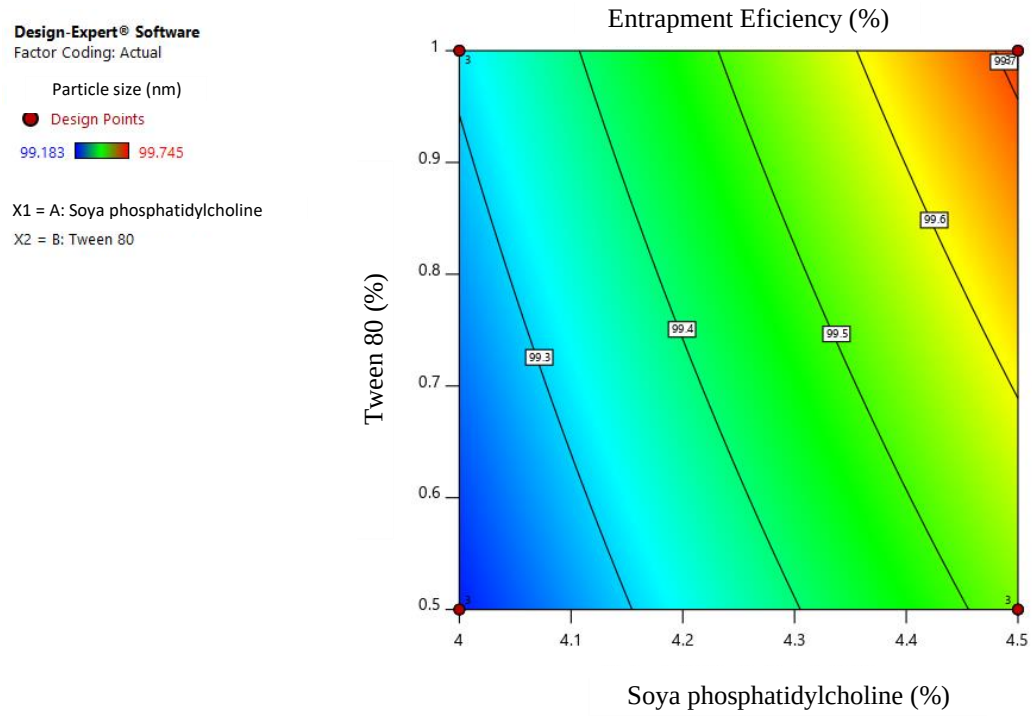

Figure 4. Graph of Entrapment Efficiency Contour Plot

After knowing the interactions that occur, then the testing process is carried out on the preparation using the optimum formula obtained to see confirmation of the response from the test results and predictions by the application. The predicted particle size is 100.068 nm, and the entrapment efficiency is 99.443%, while the results of particle measurement using PSA obtained data of 224.3 nm and the entrapment efficiency of 99.449%. The value of the entrapment efficiency test results is acceptable because it does not exceed the lower and upper limits that have been determined from the design, while the particle size value exceeds the maximum predictive value from Design-Expert but is still acceptable because it is still within the expected range of particle size values ranging from 70 -400 nm. Confirmation data can be seen in the table below.

Table 5. Confirm Design-Expert results Two-sided Confidence = 95%

Response	**Prediction Mean Value**	**Test Mean Value**	**95% *Prediction Index***	
			Low	*High*
Particle Size	100.068	224.3	64.868	135.269
Entrapment Efficiency	99.443	99.449	99.398	99.488

Characterization of Transfersome Diclofenac Sodium Optimum Formula

Entrapment Efficiency (%EE)

Entrapment efficiency is an evaluation carried out to determine the amount of drug that is adsorbed in the transfersome. The entrapment efficiency was determined by separating the adsorbed drug from the

free drug using the centrifugation method. The value of the entrapment efficiency can be calculated and the results are obtained as in Table 6.

Table 6. The Value of the Entrapment Efficiency of the Optimum Formula

Formula	Soya Phosphatidylcolin : Tween 80 (%)	Entrapment Efficiency (%)
The Optimum Formula	4.5 : 1	99.449±0.55

*Data is given in mean±SD in three replicates

It shows that the entrapment efficiency of diclofenac sodium transfersome is very good. According to research conducted by Ramadhan (2015) the comparison of the use of phosphatidylcholine and surfactants greatly affects the efficiency of the resulting transfersome entrapment. The use of high concentrations of soya phosphatidylcholine will increase the number of vesicles formed along with the increasing number of adsorbed drugs so that the value of the entrapment efficiency will also increase. This is in accordance with the entrapment efficiency values obtained in this study, that the optimum formula for transfersome Diclofenac Sodium with a concentration ratio of soya phosphatidylcholine and tween 80 of 4.5%: 1% has an entrapment efficiency value of 99.449%. This value is in accordance with the expected specifications for the value of the entrapment efficiency, which is 90-100%.

Particle Size

Particle size is one of the most important parameters in determining the optimum transfersome formula, because the vesicle size affects the encapsulation ability of drug compounds in transfersomes (Opatha et al., 2020). Determination of particle size in the optimum formula of diclofenac sodium transfersome was carried out using PSA (Particle Size Analyzer) and the results were obtained as follow:.

Table 7. Particle Size of the Optimum Formula

Formula	Soya Phosphatidylcolin: Tween 80 (%)	Particle Size (nm)
The Optimum Formula	4.5 : 1	224.3±35.05

*Data is given in mean±SD in three replicates

Based on Table 7, it can be seen that the optimum formula particle size is 224.3 nm. This result passes the predicted value specified by Design-Expert. In this study, there was an increase in the transfersome particle size during storage before particle measurement using PSA was carried out. This is explained by research conducted by Laouini et al., (2012) that during storage, nanovesicles tend to coalesce and grow into larger vesicles, but are more thermodynamically stable. However, the increase was still below the maximum target of the transfersome particle size (maximum 400 nm). Due to the ability of ultradeformable transfersomes (capable of narrowing themselves 5-10 times the size of the vesicle), so that even though they are larger in size, they are still able to penetrate easily into the stratum corneum.

The large particle size has a greater drug-loading capacity so that the amount of active substance delivered is also increasing (Rahayu, 2019).

Zeta Potential

The stability of a nanoparticle can be predicted through the magnitude of its zeta potential. According to Ratnasari and Effionora (2016), a zeta potential value greater than +30 mV or less than -30 mV indicates a high degree of stability. Because if the particles in suspension have a zeta potential value greater than -/+ 30 mV, they will repel each other so that there is no tendency to flocculate. On the other hand, if the dispersion has a low zeta potential value, it will tend to form aggregates because there can be attractive forces through the Van der Wall bond. The zeta potential value of the optimum transfersome Diclofenac Sodium formula was determined using Zetasizer.

Table 8. Zeta potential of the Optimum Formula

Formula	Soya Phosphatidylcolin: Tween 80 (%)	Zeta Potential (mV)
Formula optimum	4.5 : 1	-57.1±4.7

*Data is given in mean±SD in three replicates

Based on Table 8, it is known that the zeta potential value obtained is less than -30 mV, which is -57.1 mV. This illustrates that the optimum formula for transfersome Diclofenac Sodium has a high degree of stability and meets the criteria for the required zeta potential value. In addition, the negative zeta potential value is due to the phosphatidylcholine component used. Soya phosphatidylcholine is a zwitterionic component with an isoelectric point between 6-7, while the hydrating medium used in this study was PBS pH 7.4. At a pH higher than the isoelectric point, soya phosphatidylcholine has a negative charge (Nurmahliati et al., 2020).

CONCLUSION

The optimum formula for diclofenac sodium transfersome vesicles was a formula with a concentration of 4.5% soya phosphatidylcholine and 1% tween 80 based on Design-Expert. The characteristics of the transfersome Diclofenac Sodium vesicles with the optimum formula based on Design-Expert were transfersomes with a particle size of 224.3 nm, zeta potential -57.1 mV and entrapment efficiency of 99.449%.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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