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Research Article

OPTIMIZATION OF LUTEOLIN-LOADED TRANSFERSOME USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Objective: This research was carried out to optimize luteolin-loaded transfersome formula with independent variables such as lipid–surfactant (total lipid) concentration and luteolin concentration.

Methods: Luteolin-loaded transfersome was optimized by response surface methodology based on four parameters, namely, particle size (Z-average), polydispersity index, zeta potential, and entrapment efficiency. The transfersome formula was prepared using central composite design, and the selected independent variables were the total lipid (mixture of phospholipid and Tween 80) and luteolin concentrations. 14 formulas of luteolin-loaded transfersome were prepared by thin film hydration, followed by the sonication method.

Results: The total lipid and luteolin concentration significantly affected the entrapment efficiency only. The other parameters were not affected by a change in these variables. The optimum formula of 4.88% total lipid and 0.5% luteolin with desirability value of 0.609 conformed with the prediction parameters. Vesicle imaging using transmission electron microscopy revealed spherical particles and the occurrence of particle aggregation. The optimum formula of luteolin-loaded transfersome possessed the following characteristics: Particle size of 286.03±8.46 nm, polydispersity index of 0.480±0.013, zeta potential of -18.67±0.379 mV, and entrapment efficiency of 94.97±0.28 %. However, these values did not correspond to the predicted values and were confirmed by the low adjusted and predicted R-squared values.

Conclusion: This method can be applied to optimize the entrapment efficiency, and in the future, it can be used for further optimizing formula of transfersome by including more variables.

Keywords: Central composite design, Entrapment efficiency, Luteolin, Response surface methodology, Transfersome.

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INTRODUCTION

Luteolin is a chemical compound found in celery, chamomile flower, broccoli, and nutshell. It is a flavonoid present in plants in the aglycone form and glycoside (bonds with sugar molecules), such as cynaroside (luteolin 7-O-glycoside) [1]. It has the potential for the treatment of inflammation or gout owing to its anti-inflammatory activity and xanthine oxidase inhibition. The structure–activity relationship indicates that the presence of hydroxyl group at positions 7 and 5 in flavonoids significantly lowers the xanthine oxidase IC_{50} value [2]. However, the poor solubility and permeability of luteolin through epithelial and gastrointestinal tissue can hamper its formulation [3]. Moreover, in intestinal mucus, luteolin is glucuronidated before being released into blood circulation [4]. Therefore, drug administration through the skin is supported to overcome the metabolism problem and increase the bioavailability of the drug.

Stratum corneum is a barrier for drug penetration because it consists of high-density corneocyte layers. Drug delivery systems help enhance drug penetration through the skin. Transfersome, a lipid-based vesicle, contains lipid and a surfactant as an edge activator, which makes it deformable. Deformable transfersome can penetrate into the deeper layer of skin and deliver the drug, while conventional liposome remains in the upper layer of the stratum corneum and accumulates on the skin appendages [5]. Factors such as lipid-surfactant concentration, surfactant–lipid ratio, and drug concentration affect the characteristics of transfersome. The entrapment efficiency is increased by decreasing the drug–lipid surfactant ratio while it is decreased by increasing the drug concentration [6]. Therefore, these factors must be optimized to obtain optimum characteristics of transfersome.

Experiments were designed to determine the effect of the independent variables (factor) on the dependent variable (parameter/response) of a process or formulation [7]. Response surface methodology (RSM), one of the designs of experiments, is a powerful tool for determining the relationship between a response and a set of quantitative involved factors [7,8]. RSM is a technique used to find the optimum response by using the quadratic polynomial model [8]. The advantage of RSM is the reduced amount of experiments required, thereby reducing the cost of expensive analysis methods [9]. The application of RSM is useful for understanding or mapping a region of response surface, finding the variable level of optimum response, and selecting the process condition or formula to meet the specifications [10]. This research was carried out to optimize luteolin-loaded transfersome formula with independent variables such as lipid–surfactant (total lipid) concentration and luteolin concentration. The optimum formula was obtained from RSM by optimization of four different parameters, namely, particle size (Z-average), polydispersity index, zeta potential, and entrapment efficiency.

METHODS

Phospholiphon 90H was purchased from Lipoid (Germany), Tween 80 was obtained from Sigma (Switzerland), and luteolin was purchased from Chemfaces (China).

Experimental design

The experiment was carried out using Design Expert version 7.0.0 trial. Central composite design (CCD) was selected to determine the formula of the experiment, which involved two independent factors, namely, phospholipon 90H–Tween 80 concentration and luteolin concentration. Four parameters of transfersome were optimized, namely, particle size (Z-average), polydispersity index, zeta potential, and entrapment efficiency. The correlation values of the total lipid and luteolin concentration to the parameter values were analyzed using RSM. The

optimum formula and predicted parameter values were obtained by overlay plots of each parameter criteria by RSM.

Transfersome preparation

Luteolin-loaded transfersome was prepared by the thin film hydration method followed by sonication. This method was adopted from Zaafarany *et al*. with some modification [6]. The total lipid, consisting of phosphatidylcholine and Tween 80, was added with luteolin. It was dissolved in an organic solvent mixture of chloroform and methanol (2:1, v/v) by stirring for 30 minutes. The solution was transferred into a round bottom flask, and the organic solvent was removed by evaporation. Evaporation was conducted using a rotary evaporator (Heidolph Laborota 4011) under reduced pressure at 56°C. Thin film forming and hydration should be carried out at a temperature higher than the transition temperature (Tc). Phospholipon 90H is in a powder state at room temperature, so thin film formation occurs at a temperature above 55°C [11]. The rotation speed was controlled to below (75 rpm) at the beginning, and it was increased (up to 125 rpm) as the solvent amount decreased. The thin film was sprayed with nitrogen gas and stored in a desiccator to remove the residual solvent. Further, the film was hydrated with a phosphate buffer solution (pH = 7.4) in a rotary evaporator without pressure at 56°C for 1 hr. The film swelled and was detached from the round flask wall. The suspension was stirred for 30 minutes, and sonication was continued by using a probe sonicator for 30 minutes. All transfersome formulas were prepared by the same method according to each composition, as shown in Table 1.

Determination of particle size and polydispersity index

Particle size and index polydispersity were determined using a particle size analyzer (Malvern nanosizer and zetasizer). The transfersome suspension was dispersed in distilled water and then transferred into a disposable cuvette. The measurements were repeated for a minimum of 3 times, and the resulting particle size average, i.e., Z-average, was selected as the particle size value. The polydispersity index was determined to evaluate the particle distribution of transfersome. A polydispersity index of 0-0.05 is called monodisperse, 0.05-0.08 is almost monodisperse, 0.08-0.7 is mid-range monodisperse, and >0.7 is polydisperse [12].

Measurement of zeta potential

The zeta potential was determined using a zetasizer (Malvern zetasizer). The transfersome suspension was dispersed and then transferred into disposable cuvette. A zetasizer probe was placed into the cuvette, and care was taken to ensure no air bubbles were present in the cuvette. The zeta potential represents the stability of the suspension, and a highly positive or negative value leads to repulsion force that helps avoid particle aggregation. A zeta potential value higher than ±30 mV indicates good stability, and one higher than ±60 mV indicates perfect stability. A zeta potential value of ±20 mV indicates short-term stability, and one lower than ±5 mV indicates fast aggregation [13].

Entrapment efficiency

Entrapment efficiency was calculated in terms of the luteolin content. Luteolin-loaded transfersome was separated from the untrapped drug by centrifuging at 14000 rpm at 4°C for 1 hr. The supernatant was collected, diluted with methanol, and assayed using high-performance liquid chromatography (HPLC). HPLC was performed using the Knauer K-1001 equipped with an online solvent degasser, autosampler, and a diode array detector. The chromatographic conditions were adopted from the study by Lou *et al*. with some modifications [14]. Samples were analyzed using the C18 column (250 mm × 4.6 mm, 5 µm), with a mobile phase consisting of 5% glacial acetic acid and methanol (30:70 v/v) and a constant rate of 1 mL/minutes. The injection volume was 20 µL, and luteolin was detected at 350 nm. The entrapment efficiency of luteolin was calculated by the equation below:

$$EE(\%) = \{(Ct-Cr)/Ct\}\times 100\%$$

Where Ct is the total concentration of luteolin, and Cr is the concentration of free (untrapped) luteolin [4].

RESULTS AND DISCUSSION

Optimization of luteolin-loaded transfersome using CCD and RSM

CCD was applied to optimize the formulation. The characteristic parameters of each formula are presented in Table 1.

Total lipid and luteolin concentration showed no effect on particle size, polydispersity index, and zeta potential, but influenced the entrapment efficiency of the luteolin-loaded transfersome

The lowest and highest PDI values were 0.280 and 0.557, respectively (Table 1). The luteolin-loaded transfersome was found to be mid-range polydisperse [12]. The correlation values of the total lipid and luteolin concentration on the polydispersity index are listed in Table 2. The total lipid concentration did not affect the PDI, and the luteolin concentration had a weak effect on the PDI. This result was in contrast to that obtained by Suhaimi *et al*. (2015), which showed that a higher concentration of lipid particles and active ingredient led to a higher PDI value [15]. In this study, the PDI tends to decrease at high concentrations of the total lipid and low concentration of luteolin. The response surface of PDI based on the 2-factor interaction (2-FI) model with adjusted R-squared was 0.2110 and predicted R-squared was 0.0170. The interaction of the total lipid and luteolin concentration did not affect the PDI value and was ineffective in predicting the PDI value in the subsequent testing.

RSM revealed the effect of total lipid and luteolin concentration on particle size, polydispersity index, zeta potential, and entrapment efficiency, as shown in Table 2. A negative correlation value meant an inverse relationship.

Table 1: Parameter values of luteolin-loaded transfersome

Total lipid concentration (%)	Luteolin concentration (%)	Z-average (d.nm)	Polydispersity index (PDI)	Zeta potential (mV)	Entrapment efficiency (%)
5.00	0.75	267.3±9.7	0.327±0.033	−18.1±1.4	92.22±2.17
2.17	0.75	245.7±17.0	0.557±0.010	−20.4±3.1	85.21±4.24
3.00	1.00	375.6±4.4	0.497±0.035	−28.1±0.7	86.44±0.24
5.00	0.75	270.5±2.2	0.379±0.062	−17.3±0.5	92.39±3.33
3.00	0.50	220.0±2.9	0.474±0.034	−18.1±1.8	92.64±2.28
7.00	0.50	216.5±13.7	0.429±0.017	−18.1±1.2	96.35±0.75
5.00	1.10	210.8±5.5	0.501±0.045	−15.8±0.9	89.38±0.95
7.00	1.00	422.9±70.9	0.556±0.076	−18.4±0.5	93.24±1.46
7.83	0.75	295.3±24.5	0.492±0.042	−21.3±2.9	95.24±1.10
5.00	0.40	262.9±15.4	0.543±0.079	−24.1±1.3	96.99±1.49
2.17	0.40	260.9±9.1	0.466±0.035	−20.1±0.8	90.84±0.59
7.83	0.40	259.7±1.5	0.280±0.030	−13.6±0.6	98.62±0.84
2.17	1.10	470.4±12.1	0.543±0.023	−24.2±1.0	79.26±1.13
7.83	1.10	311.7±22.8	0.545±0.042	−17.3±0.2	93.89±2.64

Table 2 summarizes that the total lipid concentration did not affect the particle size. Luteolin concentration affected the particle size through a weak correlation. In this research, the same concentration of total lipid gave different particle size averages. Similarly, the same concentration of luteolin gave different particle size averages. The particle size average depends on the particle size reduction process. Particle size reduction using a conventional method (sonicator) has disadvantages, such as heat production, possibility of chemical degradation of drug, and non-uniform particle distribution [16]. The top-down method of nanoparticle preparation sometimes retained unreduced particles and showed bimodal size distribution [17]. Moreover, in the use of powder phosphatidylcholine, it was difficult to control thin film formation during solvent evaporation [18]. The response surface of particle size followed a cubic model with adjusted R-squared and predicted R-squared of 0.5830 and -2.3409, respectively (Table 3).

The interaction of the total lipid and luteolin concentration did not affect the particle size and was ineffective in predicting the particle size average during subsequent testing. The correlation of total lipid and luteolin concentration on the particle size parameter is shown in Fig. 1a. The plot contour of particle size shows no effect on particle size, while an increase in the luteolin concentration increased the particle size. A small particle size average was obtained from a low concentration of the total lipid and luteolin.

The correlation of the total lipid and luteolin concentration on the PDI parameter is shown in Fig. 1b. The plot contour of the PDI parameter shows that the formula of low concentration of the total lipid and high concentration of luteolin resulted in a higher PDI value and the formula of high concentration of the total lipid and low concentration of luteolin resulted in a lower PDI value. However, the range of PDI values was close to the low values when the concentration of the total lipid and luteolin was high.

Luteolin-loaded transfersome had a negative zeta potential in the range of −13.6 mV to −28.1 mV. Therefore, the transfersome suspensions had short-term stability and were partly unstable. The minimal value of the zeta potential for supporting stability is ±20 mV [13]. The transfersome composition and pH value affect the zeta potential. Acid phospholipid and phosphatidylserine are negatively charged while phosphatidylcholine is neutral at pH 7.4 [19]. Increasing the pH value will decrease the zeta potential (or cause it to be negative) because of the decrease in the H^+ concentration [20]. As seen in Table 2, the total lipid concentration had a weak effect on the zeta potential, and the luteolin concentration did not affect the zeta potential. The adjusted R-squared and predicted R-squared of the zeta potential parameter were 0.3859 and −3.9556, respectively, based on the cubic model. These values indicated that the relationship between the factor of the total lipid and luteolin concentration did not affect the zeta potential and could not predict the parameter value for a subsequent test. The plot contour of the zeta potential parameter (Fig. 1c) showed that a low concentration of the total lipid and luteolin resulted in a higher zeta potential (less stable), and a low concentration of the total lipid and high concentration of luteolin resulted in a zeta potential value close to −30 mV, which indicates better stability.

The highest entrapment efficiency was 98.62% for a high concentration of the total lipid and low concentration of luteolin, and conversely, the lowest entrapment efficiency was due to a low concentration of the total lipid and high concentration of luteolin. This result corresponded to that obtained by Colletier's *et al*. research that the total lipid concentration was directly proportional to the entrapment efficiency of protein encapsulation in liposomes [21]. An increase in the total lipid was followed by an increase in the entrapment efficiency of sodium diclofenac because the fraction of the total lipid taking part in the encapsulation reduced the efficiency [6]. As shown in Table 2, the total lipid concentration affected the entrapment efficiency. The luteolin concentration affected the entrapment efficiency in a weak correlation. A negative value in the correlation of luteolin concentration meant an inverse relationship. The response surface model of entrapment efficiency followed a quadratic equation with adjusted R-squared and predicted R-squared of0.9689 and 0.9301, respectively. This is sufficient to suggest that the model can be used to predict the value of entrapment efficiency in a subsequent test. The plot contour of entrapment efficiency (Fig. 1d) indicated a correlation between the total lipid and luteolin concentration on the entrapment efficiency parameter. A higher total lipid concentration and smaller luteolin concentration will result in higher entrapment efficiency.

Optimization of the measured parameter by variation of the formula factor was carried using Design Expert version 7.0.0 trial. The particle size, polydispersity index, and zeta potential were specified in the minimum criteria, whereas the entrapment efficiency was set out in the maximum criteria. Each parameter criterion was combined (plotted overlay) to obtain the optimum value. The optimization results of this research can be seen in Table 4.

The optimization parameter of desirability was determined by regulating the optimum input variables to obtain one or more optimal parameters. The desirability value ranged between 0 and 1, where a value of 1 is perfect, i.e., the ideal parameter value [22]. The

Table 2: Correlation value of total lipid and luteolin concentration on particle size, polydispersity index, zeta potential, and entrapment efficiency parameters

Parameters	Total lipid concentration	Luteolin concentration
Particle size (Z-average d.nm)	−0.099	0.582
Polydispersity index	−0.267	0.454
Zeta potential	0.501	−0.176
Entrapment efficiency	0.754	−0.577

Table 3: Summary of regression model of each parameter

Parameter	Regression model	SD	R-squared	Adjusted R-squared	Predicted R-squared
Particle size (Z-average d.nm)	Cubic	50.62	0.8717	0.5830	−2.3409
Polydispersity index	2-FI	0.078	0.3931	0.2110	−0.0170
Zeta potential (mV)	Cubic	2.97	0.8110	0.3859	−3.9556
Entrapment efficiency (%)	Quadratic	0.91	0.9808	0.9689	0.9301

Table 4: Characteristics of optimum formula

Objects	Total lipid (%)	Luteolin concentration (%)	Particle size (Z-average d.nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)	Desirability
Predicted	4.88	0.50	202.948	0.437498	−19.1333	95.4793	0.609
Actual			257.18±15.20	0.480±0.013	−18.67±0.38	94.97±0.28	

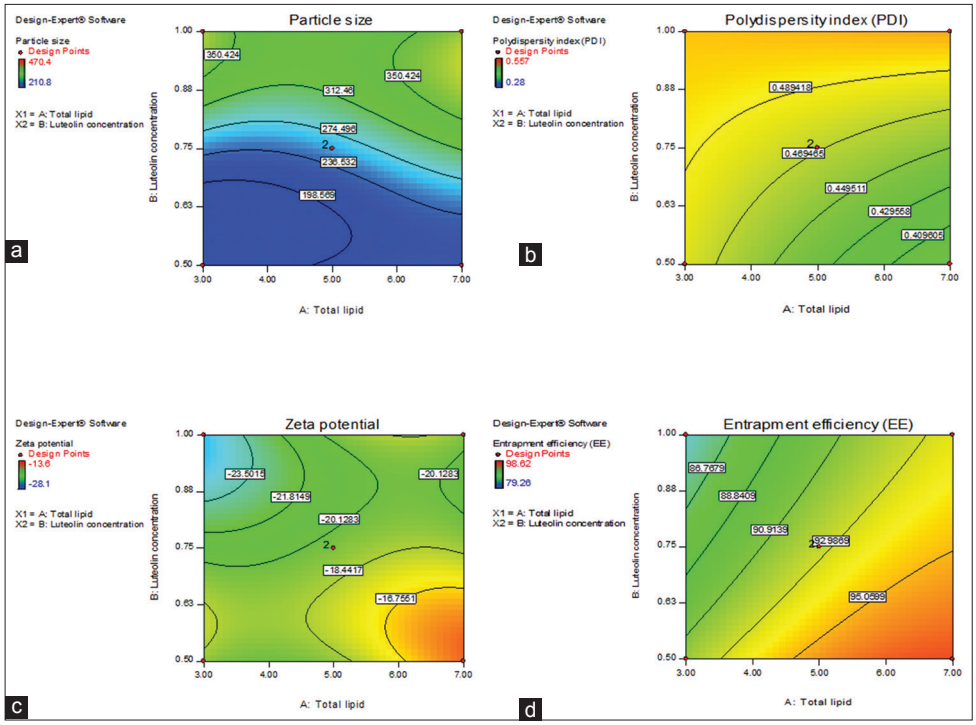

Fig. 1: Plot contour showed the interaction of total lipid and luteolin concentration on (a) particle size (Z-average d.nm), (b) polydispersity index, (c) zeta potential, and (d) entrapment efficiency parameters

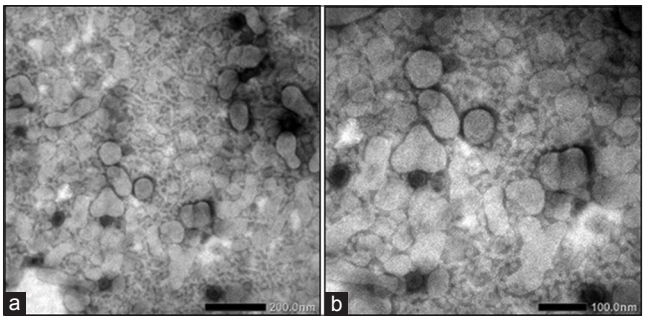

Fig. 2: Luteolin-loaded transfersome vesicle observed by transmission electron microscopy imaging (a) ×10000 and (b) ×20000 magnification

optimizing desirability of transfersome luteolin was 0.609. This value was far from the ideal value, meaning that the predicted parameters were far from the desired parameter values. Transfersome containing luteolin was prepared thrice based on the optimum formula. The parameters of each transfersome were measured, and the measured parameters were as expected in accordance with the prediction values. Transfersome containing luteolin had an average particle size and a PDI value greater than the prediction parameter value. This could be attributed to a particle size reduction, as conventional methods of size reduction may allow non-uniform size distribution [16]. The zeta potential value and entrapment efficiency were close to the prediction values. A zeta potential value of −18.67 corresponded to short-time stability. Luteolin-loaded transfersome was a light-yellow suspension. The vesicle morphology was observed using transmission electron microscopy (TEM). The TEM image showed that transfersome was a spherical vesicle and that the particles exhibited aggregation as seen in Fig. 2.

Response surface methodology is an applicable method to optimize entrapment efficiency of the formulation of luteolin-loaded transfersome.

CONCLUSION

This study to optimize Luteolin-loaded transfersome by RSM was done based on four parameters, namely, particle size (Z-average), polydispersity index, zeta potential, and entrapment efficiency from 14 formulas. However, these characteristic values of the optimum formula did not correspond to the predicted values and were confirmed by the low adjusted and predicted R-squared values. The total lipid and luteolin concentration were found to be significantly affecting the entrapment efficiency only without affecting the other parameters. This method can be applied to optimize the entrapment efficiency, and by including more variables, it can be used for further optimizing formula of transfersome.

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