ABSTRACT

Objective: The objective of this investigation was to develop and statistically optimize deformable vesicles such as transfersomes and transethosomes of Naproxen sodium by employing factorial designs through software Design expert version 12 (Box–Behnken design) for dermal delivery.

Methods: The levels of the drug, phosphatidylcholine, and span 80 (independent variables) were varied to study the influence on vesicle size and % entrapment efficiency (dependent variables) of transfersomes and for transethosomes, the levels of phosphatidylcholine, ethanol, and span 80 were selected as independent variables. Second-order quadratic polynomial equation, 2D and 3D contour plots represented the relationship between variables and desired response. The optimization process was carried out using desirability plots and point prediction techniques.

Results: Results of the present study demonstrated that optimized transfersomes and transethosomes showed vesicle sizes of 114.91 nm and 102.91 nm respectively, while entrapment efficiency of 80.11% and 86.97%, respectively. Both formulations showed high zeta potential values indicating the stability of the formulation. ANOVA statistical results showed a significant difference (P<0.05).

Conclusion: The results indicated that the independent variable plays a crucial role in optimizing a formulation that can be used for further research studies. Present preliminary study data provided strong evidence that the optimized deformable vesicular formulations through box Behnken factorial design can be a potentially useful drug carrier for naproxen sodium dermal delivery with minimum vesicle size and efficient entrapment efficiency.

Keywords: Naproxen sodium, Transfersomes, Transethosomes, Factorial design, Box Behnken design, Transdermal
Experimental methods

Method of preparation of naproxen sodium loaded transfersomes

Rotary evaporation-sonication method

Egg Phosphatidylcholine and edge activator (surfactant) were dissolved in chloroform and methanol in a round bottom flask (RBF). The ratio of chloroform to methanol was 2:1 v/v. through rotary flask evaporator solvent evaporation results in the formation of a thin film. water is used as a hydrating medium for 1 h at room temperature for hydration of formed film, which resulted in multilamellar film vesicles. These vesicles are converted into small unilamellar vesicles through ultrasound cavitation and probe sonicator [12, 13].

Method of preparation of naproxen sodium loaded transethosomes

The cold method is used for preparing transethosomes, Egg Phosphatidylcholine was dissolved in ethanol in a conical flask with constant stirring at 700 rpm. The temperature of this alcoholic mixture was maintained at 30 ° C. Drug and Span 80 were dissolved in water. This aqueous phase was then added to the alcoholic phase slowly in a fine stream with constant stirring at 700 rpm in a closed vessel. It was stirred for an additional 5 min. The system was kept at 30 ° C throughout the preparation. Size reduction was done by probe sonication for 5 min at RT. The selection of a method for the preparation of transethosomes was based on percent entrapment efficiency, stable transethosomal formulations can be achieved through the cold method [14].

Experimental factorial design: box behenken

Optimization of process parameters variables

The process of rotary vacuum evaporation were varied by varying the process variables to investigate their effect on the characteristics of carrier systems. The process variables Temperature and Rotation per minute (RPM) were varied based on 2 factors, 3 levels of general factorial design, and experimental trials were performed on all batches and based on the quality of film produced, the process was optimized [15, 16].

Experimental design for the formulation of naproxen sodium loaded transfersomes

The optimization of Naproxen Sodium Transfersomes was done by Design Expert, Version 12, Stat-Ease Inc. Minneapolis, USA using three factors at three levels. It provides a rationale for understanding the possible interaction(s) among both independent and dependent variables and helps in selecting 'optimum' formulation in lesser experimental time. The design will help to identify the positive or negative effect of a different variable on desired response. (X1) Phosphatidylcholine (mg) was taken as the first independent variable, (X2) Span 80 (mg) as the second independent variable and (X3) Naproxen sodium (mg) was selected as the third independent variables for Transfersomes. These variables varied at three levels, low level (-1), medium level (0), and high level (+1). Vesicle size (nm) (Y1) and % entrapment efficiency (Y2) were selected as dependent (Response) variables [17].

Experimental design for the formulation of naproxen sodium loaded transethosomes

Phosphatidylcholine (mg) (X1) was taken as the first independent variable, Ethanol % (X2) was selected as the second independent variable, and (X3) Span 80 (mg) as the third independent variables. These variables varied at three levels, low level (-1), medium level (0), and high level (+1). The amount of drugs were kept constant. Vesicle size (nm) (Y1) and % entrapment efficiency (Y2) were selected as dependent (Response) variables.

Characterization of deformable vesicles

Vesicle size and zeta potential

The formulated vesicles (0.1 ml) were diluted (100 times) with phosphate buffer (pH 6.8) for vesicle size and zeta potential analysis. Each formulation sample (0.1 ml) was diluted (100 times) with phosphate buffer (pH 6.8), and the size analysis was performed at 25 °C with an angle of detection of 90 °C. The mean vesicle size and zeta potential were evaluated using a Particle size analyzer and Zeta sizer (Horriba Instruments Ltd). The optimized formulation was characterized by a zeta potential value to reveal the stability of the formulation [18].

Determination of entrapment efficiency

For estimation of the entrapped drug in the prepared vesicle formulation, the unentraped drug is separated by cooling centrifugation (7000×g) at 4 °C using a cooling centrifuge for 60 min. Then washing through 40 ml PBS (pH 7.4). The supernatant is collected and the quantity of the unentraped drug was estimated by UV spectrophotometer at 271 nm, formula for calculating % entrapment efficiency is as follows [19].

\[
\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug added} - \text{Amount of unentrapped drug}}{\text{Drug added x 100}}
\]

RESULTS AND DISCUSSION

Optimization of process parameters variables

During experimental optimization, it is seen that lower speeds of instruments increase the time of contact of the film with the hot water used for the formation of a film, at higher speeds, sufficient time was not available for the lipid to form film and liquid to gel transitions of the lipid is difficult to happen this improper distribution of heat leads to the formation of the uneven film. The hydration of vesicles was affected by different temperatures. When the high temperature is selected for the process, the stability and appearance of vesicle film are not good however, at low temperature a thin film formed, which was uniform and translucent in appearance. Hence, the most appropriate temperature is 50 °C. Finally, it was observed that at 50 °C temperature and 80 rpm a thin film formed, which was uniform, good in appearance with good stability.

Table 1: Effect of rotation speed and temperature for hydration on the trial batches

| Formulation | (X1) temp (°C) | (X2) Rpm | Condition of formed film* |
|-------------|---------------|----------|---------------------------|
| Trial 1     | 50            | 100      | Thin lipid film formed but not rigid and uniform in appearance |
| Trial 2     | 70            | 100      | A bumpy film with entrapped air bubbles |
| Trial 3     | 50            | 80       | The rigid and translucent lipid film was formed with good stability |
| Trial 4     | 60            | 100      | A bumpy film with entrapped air bubbles |
| Trial 5     | 60            | 80       | The rigid film was formed, but less stability |
| Trial 6     | 50            | 60       | Thin lipid film formed but not rigid and uniform in appearance |
| Trial 7     | 70            | 80       | Film not formed |
| Trial 8     | 60            | 60       | Thin lipid film formed but not rigid and uniform in appearance |
| Trial 9     | 80            | 80       | Film not formed |

*Average of three determinations±standard deviation
Vesicle size analysis

Vesicle size plays an important role in skin permeation. Smaller vesicular size (200 nm) facilitates the deformable vesicles to pass through the small pores of the skin leading to more enhanced skin permeation. The average vesicle size of all 15 experimental runs is 121.62 nm, with values lying between the minimum and maximum of 63.21±2.34 nm (Formulation 5) and 199.36±1.52 nm (Formulation 7), respectively.

Vesicle size second-order quadratic polynomial equation

\[ Y_1 = 76.89 + 16.47X_1 + 12.80X_2 - 21.83X_3 - 1.19X_1X_2 + 2.38X_1X_3 - 14.72X_2X_3 + 8.84X_1^2 + 55.62X_2^2 + 18.55X_3^2 \]

Where \( Y_1 \) is Response 1: Vesicle size (nm), \( X_1 \) is Phosphatidylcholine (mg), \( X_2 \) is Ethanol (%), and \( X_3 \) is Span 80 (mg).

As per the Quadratic second-order polynomial equation, the phosphatidylcholine is showing a positive effect and the surfactant is exhibiting a negative effect on vesicle size. It was observed that the vesicle size of transethosomes was increased with an increase in the concentration of phosphatidylcholine. The result shows that vesicle size was increased from 63.21±2.34 nm (formulation 5) to 170.12±1.56 nm (formulation 11) when phosphatidylcholine was increased from 70 mg (formulation 5) to 110 mg (formulation 11). Surfactant also plays a very important role in vesicle size as edge activator. The result shows that Span 80 presented an inverse effect on vesicle size. Vesicle size of prepared transethosomes was decreased on increasing the Span 80 from 15 mg to 25 mg. Formulation 7 having Span 80 15 mg presented vesicles size of 199.36±1.52 nm while formulation 5 presented vesicles size of 63.21±2.34 nm which comprised of 25 mg of Span 80. Similarly, formulation 15 (Span 80, 15 mg) presented vesicles size of 145.86±1.28 nm while formulation 4 (Span 80, 25 mg) exhibited vesicles size of 101.11±1.49 nm [20, 21].

Entrapment efficiency analysis

The entrapment efficiency of all the formulation was lying in between a minimum and maximum value of (Formulation 1) 55.23% and (Formulation 15) 96.14±0.63% with the average value of 73.21 %

Entrapment efficiency second-order quadratic polynomial equation

\[ Y_2 = 74.02 + 6.147X_1 - 10.31X_2 - 8.33X_3 - 1.94X_1X_2 + 2.79X_1X_3 + 2.85X_2X_3 - 2.15X_1^2 - 1.89X_2^2 + 2.52X_3^2 \]

Where \( Y_2 \) is Response 2: Entrapment efficiency (%), \( X_1 \) is Phosphatidylcholine (mg), \( X_2 \) is Ethanol (%) and \( X_3 \) is Span 80 (mg).

A positive value before a factor in the response equation denotes a positive impact that favors optimization, while a negative value indicates a negative impact between the factor and the response. Results show that the concentration of phosphatidylcholine is showing a positive effect on entrapment efficiency while the other two variables ethanol and Span 80 were found to exhibit a negative effect on the entrapment efficiency of the drug. The concentration of phosphatidylcholine was showing a positive effect.

Formulation 13 containing 110 mg of Phosphatidylcholine had exhibited entrapment efficiency of 88.62±0.35 % on comparison with formulation 1 possessing 80 mg of Phosphatidylcholine showing an entrapment efficiency of 55.23±0.23%. The gradual increase of entrapment efficiency with an increase in the concentration of phosphatidylcholine is because of the inherent lipophilic character of naproxen sodium as the lipophilic drug would be attracted towards the lipophilic phase and get deposited over there. Ethanol presented an inverse effect on the entrapment efficiency of naproxen sodium in transethosomes. Formulation 15 (ethanol 20%) presented an entrapment efficiency of 96.14±0.63%, while formulation 1 having ethanol 40% presented an entrapment efficiency of 55.23±0.23%. Similar results were obtained for formulation 13 and 9. Formulation 1 (ethanol 20%) presented entrapment efficiency of 88.62±0.35% and formulation 9 (ethanol 40%) presented entrapment efficiency of 58.87±0.65%. This could be due that at a higher ethanol percentage; vesicles become leakier [22]. Surfactant also plays a very important role in Entrapment efficiency as edge activator. Entrapment efficiency decreased on increasing the concentration of Span 80 Formulation 5 (Span 80, 15 mg) presented entrapment efficiency of 96.14±0.63% while formulation 5 (Span 80, 25 mg) presented entrapment efficiency of 57.39±2.10%. Similar results were obtained between formulation 14 (entrapment efficiency 96.14±0.63%) and formulation 9 (entrapment efficiency 58.87±0.65%). The decrease in entrapment efficiency with an increase in the concentration of Span 80 is because of the coexistence of micelle structure with vesicles in the formulation; micelles usually exhibit low entrapment efficiency as compared to vesicles [23].

Zeta potential measurement
Zeta potential of prepared transethosomes is in the range of 20±0.31 mV (Formulation 1) and 34±1.66 mV (Formulation 15) which suggest relative stability of vesicles with minimum aggregation properties. The highest Zeta potential of vesicles is -34±1.66 mV (Formulation 1). Analysis of variance (ANOVA) was applied to determine the statistical significance and the magnitude of the main effects of each variable and their interactions. Counterplots are generated for independent factors. The ANOVA table establishes the sufficiency of the model (i.e., p<0.05). The result of the p-value is less than 0.05 for all the response factors signifying that the models are significant. Comparison of the experimental and predicted responses. These data showed that most of the predicted values are nearly similar to the experimental values. These indicated the excellent ability of the experimental design employed for the optimization of transethosomal formulation of Naproxen sodium. The predicted R² values were in agreement with the adjusted R² values in all responses. The relationship between the dependent and independent variables was further elucidated using 2D contour plots and 3D response surface plots. Desirability and graphical optimization technique were utilized for generating formulation with the desired responses. The 3D response surface plots are useful to understand the effect of interactions between the factors on a single response.

ANOVA for vesicle size (Response 1)
The result of P-values is 0.0001 (Significant) the Model F-value is 1811.82 showing that the model is significant. P-values less than 0.05 confirm the significance of the model. Model is not significant when values are greater than 0.1000. The Predicted R² of 0.9955 agrees with the Adjusted R² of 0.9991, the difference is less than 0.2.

ANOVA for entrapment efficiency (%) (Response 2)
The result of P-values is 0.0001 (Significant) the model F-value is 1202.04 showing that the model is significant. P-values less than 0.05 confirm the significancy of the model. Model is not significant when values are greater than 0.1000. The Predicted R² of 0.9944 agrees with the Adjusted R² of 0.9987; the difference is less than 0.2

Selection of the optimum formula
Further in this study, the point prediction method of Box-Behnken design was utilized for the optimization of transethosomes formulation. The optimized formula desirability function comes higher ie near to 1 confirms the suitability of the formulations. Further, the zeta potential (fig.) value of optimized formulation was found to be -35 mV; the optimized formulation so produced will be further evaluated for vesicles morphology, in vitro release study, skin irritation study, and in vivo study.
Design-Expert Version 12 software. The best fit model was decided on the high values of multiple correlation coefficient ($R^2$), adjusted $R^2$, and predicted $R^2$ and low values of standard deviation (SD). To evaluate the effect of variables on each response, the responses were analyzed to multiple linear regression analysis to generate a second-order quadratic polynomial equation.

### Vesicle size analysis

The average vesicle size of all 15 experimental runs was found to be 140.20 nm, with values lying between the minimum and maximum of 114.49±1.54 nm (Formulation 2) nm and 174.65±0.89 nm (Formulation 10), respectively.

### Table 3: Experimental value obtained through point prediction method

| Composition                  | Optimized level | Response      | Predicted value | Experimental value* |
|------------------------------|-----------------|---------------|-----------------|---------------------|
| Phosphatidylcholine (mg)     | 90.07           | Vesicle size (nm) | 103.19          | 102.91              |
| Ethanol (%)                  | 25.07           | Entrapment efficiency | 86.62          | 86.97               |
| Span 80 (mg)                 | 16.53           | (%)           |                  |                     |

### Table 4: Experimental runs, independent variables, and measured response of 3³ full factorial experimental design for transfersomes

| Run | X₁ | X₂ | X₃ | Y₁ | Y₂ |
|-----|----|----|----|----|----|
| 1   | 35 | 80 | 24 | 146.38±0.23 | 78.0±0.65 |
| 2   | 35 | 60 | 30 | 114.49±1.54 | 75.9±1.53 |
| 3   | 35 | 18 | 60 | 136.54±0.58 | 59.1±1.25 |
| 4   | 20 | 60 | 24 | 134.21±1.56 | 68.7±1.56 |
| 5   | 20 | 80 | 30 | 123.32±1.11 | 89.6±0.89 |
| 6   | 50 | 80 | 18 | 148.67±2.54 | 51.4±1.65 |
| 7   | 20 | 100| 24 | 139.34±0.78 | 77.2±0.23 |
| 8   | 35 | 80 | 24 | 146.38±0.56 | 74.3±1.65 |
| 9   | 35 | 20 | 24 | 146.38±0.10 | 76.4±0.45 |
| 10  | 20 | 80 | 18 | 174.65±0.89 | 51.1±0.82 |
| 11  | 35 | 100| 30 | 119.23±0.65 | 89.9±1.11 |
| 12  | 50 | 100| 24 | 144.23±0.62 | 67.6±1.53 |
| 13  | 50 | 60 | 24 | 126.16±0.35 | 56.3±0.45 |
| 14  | 50 | 80 | 30 | 135.33±1.52 | 70.8±2.54 |
| 15  | 35 | 100| 18 | 167.77±0.45 | 53.4±0.89 |

*Average of three determinations±standard Deviation

### Vesicle size quadratic second-order polynomial equation

\[ Y_1 = 146.38 - 2.14125X_1 + 7.39625X_2 - 1.69075X_3 + 3.235X_1X_2 + 9.4975X_1X_3 + 0.295X_2^2 - 10.69X_3^2 - 1.1825X_1X_3 \]

Where $Y_1$ is Response 1: Vesicle size (nm), $X_1$ is Naproxen sodium (mg), $X_2$ is Phosphatidylcholine mg, $X_3$ is Span 80 (mg)

As per the second-order quadratic polynomial equation, the phosphatidylcholine is showing a positive effect on vesicle size, and surfactant and drug concentration were exhibiting a negative effect on vesicle size. The result showed that the size of vesosomes was increased with an increase in the concentration of phosphatidylcholine formulation 15 with phosphatidylcholine (100 mg) has a high vesicle size of 167.77±0.45, and formulation 10 with phosphatidylcholine (80 mg) has a high vesicle size of 174.65±0.89. Surfactant also plays a very important role in vesicle size as edge activator. It was observed that Span 80 presented an inverse effect on vesicle size formulation 10 with span 80 (18 mg) has a high vesicle size of 174.65±0.89, and formulation 15 with span 80 (18 mg) has a high vesicle size of 167.77±0.45. As the drug concentration reaches the lowest vesicle size becomes highest for example formulation 10 with Naproxen sodium (20 mg) has a high vesicle size of 174.65±0.89.

The surfactants destabilize vesicular bilayer reduces interfacial tension hence enhances bilayer elasticity, hence leads to a decreased particle size. For skin delivery, small particle size is of importance for skin penetration and drug deposition [24]. New research shows a mechanism picture of vesicle solubilization formation and concluded that surfactant-containing bilayers, at certain lipid/surfactant ratio, spontaneously un/curve and open or close to optimizing edge tension [25]. In transfersomes incorporation of edge activators in low concentration results in vesicle size growth [26].

### Entrapment efficiency analysis

The entrapment efficiency of all formulations found in between a minimum and maximum value of 51.1±0.82 % and 89.9±1.11 % with the average value of 69.36 %.

### Entrapment efficiency second-order quadratic polynomial equation

\[ Y_2 = 76.2833 - 5.06125X_1 + 3.51625X_2 + 13.8925X_3 + 0.7X_1X_2 - 4.7925X_1X_3 + 4.9375X_2X_3 - 6.32917X_1^2 - 2.40917X_2^2 - 4.186667X_3^2 \]

Where $Y_2$ is Response 2: Entrapment efficiency (%), $X_1$ is Naproxen sodium (mg), $X_2$ is Phosphatidylcholine (mg), $X_3$ is Span 80 (mg).

Results showed that the concentration of phosphatidylcholine and span 80 were showing a positive effect on entrapment efficiency, while the naproxen sodium was found to exhibiting a negative effect on the entrapment efficiency of the drug. The result shows that the entrapment efficiency of transfersomes was increased with an increase in the concentration of phosphatidylcholine formulation 11 with phosphatidylcholine (100 mg) shows high entrapment efficiency of 89.9±1.11. Surfactant also plays a very important role.
in Entrapment efficiency as edge activator. Entrapment efficiency increases on increasing the concentration of Span 80 Formulation 11 (Span 80 30 mg) presented high entrapment efficiency of 89.96±1.11% while formulation 5 (Span 80 30 mg) presented high entrapment efficiency of 89.66±0.89%. As Naproxen sodium concentration decreases entrapment efficiency increases. As the drug concentration reaches the lowest entrapment efficiency becomes highest for example, formulation 5 with Naproxen sodium (20 mg) has a high Entrapment efficiency of 89.66±0.89%

Zeta potential measurement

Zeta potential values of prepared transfersomes is in the range of -22±0.54 mv (Formulation 10) and -37±1.65 mv (Formulation 11) which suggest relative stability of vesicles with minimum aggregation properties. Highest Zeta potential of vesicles is -37±1.654 mv (Formulation 11).

Analysis of variance (ANOVA)

The ANOVA table confirms the adequacy of the model (i.e., p<0.05). The p-value is less than 0.05 for all the response factors indicating that the models are significant. These data showed that most of the predicted values were close to the experimental values. These indicated the excellent prognostic ability of the experimental design employed for the optimization of transfersomal formulation of Naproxen sodium. The predicted R² values were in reasonable agreement with the adjusted R² values in all responses.

ANOVA for vesicle size (Response 1)

The result of P-values is 0.0002 (Significant) the Model F-value is 53.15 showing that the model is significant. P-values less than 0.05 confirm the significance of the model. Model is not significant when values are greater than 0.1000. The Predicted R² of 0.8345 agrees with the Adjusted R² of 0.9710, the difference is less than 0.2 [27, 28].

ANOVA for entrapment efficiency (%) (Response 2)

The result of P-values is 0.0003 (Significant) the Model F-value is 45.06 showing that the model is significant. P-values less than 0.05 confirm the significance of the model. Model is not significant when values are greater than 0.1000. The Predicted R² of 0.8479 agrees with the Adjusted R² of 0.9659, the difference is less than 0.2

Selection of the optimum formula

Further in this study, the point prediction method of Box-Behnken design was utilized for the optimization of transfersomes formulation. The optimized formula desirability function comes higher i.e near to 1 confirms the suitability of the formulations. Further, the zeta potential value of optimized formulation was found -37 mV, respectively. The optimized formulation so produced will be further evaluated for vesicle morphology, in vitro release study, skin irritation study, and in vivo study.

Table 5: Experimental value obtained through point prediction method

| Composition       | Optimized level | Response     | Predicted value | Experimental value* |
|-------------------|-----------------|--------------|-----------------|---------------------|
| Naproxen sodium (mg) | 20.74           | Vesicle size (nm) | 114.33          | 114.91              |
| Phosphatidylcholine (mg) | 60.70          | Entrapment efficiency (%) | 79.69           | 80.11               |
| Span 80 (mg)       | 29.86           |              |                 |                     |
CONCLUSION

Present study utilizes a factorial design (Box Behnken) for the preparation of naproxen sodium transfersomes and transethosomes with desired characteristic responses. The factorial design describes the relationship between an experimental response and a set of input variables. Optimization of vesicles signifies a point prediction model for the selection of variable ranges to achieve the expected desired outcome and responses. Within a small experimental trial setup, the desired responses can be achieved by a systematic experimental formulation approach for establishing a mathematical trend in the experimental design. In our study, optimized formulations show minimum vesicle size, good entrapment efficiency, and optimum zeta potential, which justify the usefulness of drug carrier for dermal delivery of naproxen sodium.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
The authors declare no conflict of interest in this work.

REFERENCES
1. Benson H. Transfersomes for transdermal drug delivery. Expert Opinion Drug Delivery 2006;3:727-37.
2. Priya K, Kumar V, Damini V, Eswar K, Reddy KR, Brito Raj S, Sucharitha P. Some: a review on composition, formulation methods and evaluations of different types of "somes" drug delivery system. Int J App Pharm 2020;12:7-18.
3. Pandey P, Pancholi S. Nanocarriers: a novel treatment approach for arthritis. Int J Pharm Sci Res Vol 2013;4:4165-74.
4. Kumavat S, Chaudhari Y, Borole P. Transfersomes: a promising approach for transdermal drug delivery system. Asian J Pharm Sci 2013;3:1-17.
5. Meeei M, Gulasekharam V. Liposomes—a selective drug delivery system for the topical route of administration lotion dosage form. Life Sci 1980;26:1473–7.
6. Verma D, Verma S, Blume G. Particle size of liposomes influences dermal delivery of substances into the skin. Int J Pharm 2003;258:141–51.
7. Manosroi A, Manosroj P, Manosroi J. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethyl ammonium. Int J Pharm 2008;360:156–63.
8. G Cec. Transfersomes, liposomes, and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 1999;6:13:257-388.
9. Maghraby G, Williams A, Barry A. Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes. Int J Pharm 2004;276:143-61.
10. E Touitou, M Alkabes, N Dayan. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. PharmRes 2000;15:403-18.
11. Ekayed M, Abdallah O, Naggar V, Khalafalah V. Lipid vesicles for skin delivery of drugs: reviewing three decades of research. Int J Pharm 2007;332:1-16.
12. Chaurasia G, Lariya N. A comparative assessment of vesicular formulations: transfersomes and conventional liposomes loaded ivabradine hydrochloride. Int J Appl Pharm 2020;12:51-5.
13. Shaji J, Vinor M. Novel double-loaded transfersomes: evidence of superior anti-inflammatory efficacy—a comparative study. Int J Curr Pharm 2014;6:16-25.
14. Shaji J, Bajaj R. Formulation development of 5-fluorouracil transethosomes for skin cancer therapy. Int J Pharm 2017;11:453-64.
15. Shaji J, Maria Lal. Preparation, optimization, and evaluation of transferosomal formulation for enhanced transdermal delivery of a cox-2 inhibitor. Int J Pharm Pharm Sci 2014;6:467-77.
16. Vyas P, Vyas P, Raval D, Paghdar P. Development of topical niosomal gel of benzoyl. Nanotechnology 2011. https://doi.org/10.5402/2011/503158.
17. Sara M Soliman, Nevine S Abdelmalak, Omaira N. Novel non-ionic surfactant proniosomes for transdermal delivery of lacticacid: optimization using 23 factorial design and in vivo evaluation in rabbits. Drug Delivery 2016;12:1608–22.
18. Mohanty D, Rani M, Haque M. Preparation and evaluation of transdermal-naproxen niosomes: formulation optimization to preclinical anti-inflammatory assessment on the murine model. J Liposome Res 2019;30:1-11.
19. Alima S, Kassem A, Bashaa M, Salama A. Comparative study of liposomes, ethosomes, and transfersomes as carriers for enhancing the transdermal delivery of diflunisal: in vitro and in vivo evaluation. Int J Pharm 2019;30:293–303.
20. Touitou E, Dayan N, Bergelson L. Ethosomes–novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Controlled Release 2000;65:403-18.
21. Dubey V, Mishra D, Dutta T. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Controlled Release 2007;121:148–54.
22. Ahad A, Agil M, Kohli K. Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: statistical optimization characterization and pharmacokinetic assessment. Int J Pharm 2013;443:26–38.
23. El Zaafarany GM, Awad GA, Holayel SM. Role of edge activators and surface charge in developing ultra deformable vesicles with enhanced skin delivery. Int J Pharm 2010;397:164–72.
24. Chen G, Li D, Jin Y, Zhang W, Teng L. Deformable liposomes by reverse-phase evaporation method for enhanced skin delivery of (þ)-catechin. Drug Dev Ind Pharm 2013;40:260-5.
25. Simoes SI, Marques CM, Cruz ME, Cevc G, Martins M. The effect of cholate on solubilization and permeability of simple and protein-loaded phosphatidylcholine/sodium cholate mixed aggregates designed to mediate transdermal delivery of macromolecules. Eur J Pharm Biopharm 2004;58:509-19.
26. Van den Bergh BA, Wertz PW, Junginger HE, Bouwstra JA. The elasticity of vesicles assessed by electron spin resonance, electron microscopy, and extrusion measurements. Int J Pharm 2001;1:13-24.
27. Armatazaka Z, Sulaiman TN, Zulkarnain AK. Optimization and characterization of PEG-PCL-PEG triblock copolymer as a carrier of drug-using full factorial design. Int J Curr Pharm Res 2020;11:65-71.
28. Chuo W, Kuang Y, Huang YT, Shan C. Statistical optimization and stability study of the quercetin-loaded microemulsion. Int J Pharm Pharm Sci 2020;13:13-25.