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# ETHOSOMAL AND TRANSFERSOMAL GEL OF ADAPALENE FOR THE TREATMENT OF ACNE : A COMPARATIVE STUDY

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## Abstract

The aim of the present study is to prepare and characterize transfersomal gel and ethosomal gel of adapalene for the treatment of acne and compare which among the two shows better results. Transfersomes and ethosomes are optimized by using design expert software. The trials were conducted by using the effect of independent factors like the amount of phospholipid, cholesterol, tween 80 and sodium deoxycholate for transfersome and the amount of soya phosphatidylcholine, ethanol, cholesterol for ethosomes.

The optimized transfersomes had a particle size of 128.8 nm, PDI of 0.364, zeta potential of -40.3 and entrapment efficiency of 68.33 %. *In vitro* drug release study of the gel containing optimized transfersome exhibited 84.88 % drug release. *Ex vivo* study exhibited 67.98 % of the drug release. The optimized ethosomeshad a particle size of 192.3 nm, PDI of 0.523, zeta potential of -49.5 and entrapment efficiency of 63.4%. *In vitro* drug release study of the gel containing optimized ethosome exhibited 79.92 % drug release. *Ex vivo* study showed 62.87% of the drug release. The above studies conclude that the transfersomal gel was found to be better for topical application as compared to that of ethosomal gel for acne treatment.

***Key words:*** Transfersome, Ethosome, Adapalene, acne, gel.

## Introduction

Acne is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production. It is characterized by both inflammatory and non-inflammatory lesions. Inflammatory lesions include papules, pustules and nodules and non-inflammatory lesions include open and closed comedones. Staphylococcus epidermidis and Propionibacterium acnes are common pus-forming microbes that are responsible for the development of various forms of acne vulgaris (Khan, N.R. *et al*., 2018). Acne is a most common long-term skin disease which occurs when the hair follicles get clogged with dead skin cells, dirt and oil from the skin. They are characterized by blackheads, whiteheads, pimples, inflammatory papules, pustules, nodules and cysts which may lead to scarring and pigmentary changes (Lynn, D. *et al*., 2016). One of the major symptoms in acne is seborrhea, which has a sign like greasy, itchy, red, scaly and inflamed skin seen mainly in the areas of the skin rich in oil-producing glands and often affects the scalp, face and chest (Fabbrocini, G. *et al*., 2010).

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The main topical acne therapy is by retinoids, benzoyl peroxide (antimicrobials) and antibiotics. These are proven effective by applying at the sites of the acne affected area and they are capable of preventing the formation of new lesions (Su Youn, 2016). Adapalene is a synthetic naphthoic acid derivative with retinoid activity. The chemical name of adapalene is 6[3-(1-adamantyl)-4-methoxyphneyl]-2naphthoic acid. Adapalene acts by modulating cellular keratinization and inflammatory process. The anti-inflammatory activity is due to the inhibition of the lipooxygenase activity and also due to the oxidative metabolism of arachidonic acid. To obtain the sustained release pattern they were incorporated into the vesicular systems. The vesicular systems used in this current study were transfersomes and ethosomes.

Transfersomes are defined as ultra-deformable, self-

adaptable, flexible elastic bilayer vesicles which are composed of phospholipids and edge activators like surfactants. They are stress adaptable and can passes through the pores of skin which are smaller than their size as these can squeeze through the pores by deforming themselves and then reform after passing through pore (Garg, V. *et al*., 2016). Transfersomes are stress-responsive complex aggregate having a complex lipid bilayersurrounding the aqueous core (Zhang, Y.T. *et al*., 2014). Ethosomes are soft, malleable lipid-based vesicular system consisting of a phospholipid, high concentration of ethanol and water (Iizhar, S.A. *et al*., 2016). It consists of amphipathic phospholipids arranged in one or more concentric bilayers enclosing numerous aqueous compartments (Mohanty, D. *et al*., 2016). The high flexibility of ethosomes is due to the presence of ethanol which allows the vesicles to readily squeeze through the skin pores, which are much smaller than their diameters, thereby disturbing skin lipid bilayer organization and thereby enhancing the therapeutic efficacy (Verma, P. 2012).

## Material and Methods

Adapalene was obtained from Saiyasika Biochem, Chennai. Soya lecithin was obtained from Himedia Lab Pvt. Ltd, Mumbai. Cholesterol, diethyl ether, chloroform, tween 80, sodium deoxycholate, dimethyl sulfoxide, propylene glycol, carbopol 934 was obtained from Loba Chemie Pvt. Ltd, Mumbai. Soya phosphatidylcholine was obtained from Sigma Aldrich, USA. Ethanol was obtained from Nice Chemical Pvt. Ltd. Kerala. All the chemicals used in the formulation were of analytical grade.

### Formulation of adapalene loaded transfersome

Transfersomes were formulated by a reverse phase evaporation method. An accurately weighed amount of soya lecithin and cholesterol were taken in a clean beaker and dissolved in the solvent mixture containing diethyl ether and chloroform in the ratio 3:1. Tween 80 was added to the same beaker and dissolved in the above solvent mixture. The beaker was kept at room temperature for 24 hrs. for the formation of a thin film. 0.1% drug solution of adapalene was poured onto the film and was stirred at 700 rpm for 1 hr. The solution was sonicated by probe sonicator (5 min, 1 cycle, 5 pulses, 3s interval, 50% amplitude at 4ºC). The film was hydrated using sodium deoxycholate solution. It was further was sonicated to obtain a transferosomes. 5% Dimethyl sulfoxide (DMSO) was added and the suspension was stored at 4ºC (Thakur, N., 2018).

### Formulation of adapalene loaded ethosome

Adapalene loaded ethosomes were prepared by the cold method. In this method, accurately weighed amount of soya phosphatidylcholine, cholesterol and drug was first dispersed in 45% ethanol and stirred at 700 rpm using magnetic stirrer at room temperature until the colloidal suspension was obtained. Once the colloidal suspension is formed, 5ml propylene glycol was added and the mixture was kept stirring at 40°C. In a separate beaker, water was warmed at 40°C. Once both the solutions reach 40°C, the aqueous phase was added to the organic phase with the continuous stirring at 700 rpm for 1 hr. The temperature was maintained constant for the entire process. It was sonicated using probe sonicator at 4°C (3 min, 3 cycles, 2 pulses, 3s interval, 50% amplitude). The formulation was stored at 4ºC (Patel, K.K. *et al*., 2012).

**Table 1:** Factors and their corresponding levels used for transfersomes.

| Factor | Name | Level (-1) | Level (+1) |
|---|---|---|---|
| A | Soya Lecithin (mg) | 150 | 400 |
| B | Cholesterol (mg) | 25 | 100 |
| C | Tween 80 (mg) | 10 | 30 |
| D | Sodium Deoxycholate (mg) | 10 | 100 |

### Design of Experiment

A central composite design, response surface method (RSM) with randomized subtype was used to study the overall influence of independent variables on the formulation of the vesicles. The independent variables selected for optimization of transfersomes were soya lecithin, cholesterol, tween 80 and sodium deoxycholate. (Table 1). The independent variables selected for optimization of ethosomes were the percentage of soya phosphatidylcholine, cholesterol and ethanol. (Table 2) The dependent variables for transfersomes and ethosomes were particle size, PDI, zeta potential and entrapment efficiency.

The four independent variables were selected at two levels, coded as -1 (low) and +1 (high) resulting in 24 factorial design of 30 possible combinations of the selected variables for transfersomes and 20 possible combinations for ethosomes.

### Physicochemical characterization

- Particle size, PDI and zeta potential:

Particle size, PDI and zeta potential analysis was

**Table 2:** Factors and their corresponding levels used for ethosomes.

| Factor | Name | Level (-1) | Level (+1) |
|---|---|---|---|
| A | Soya Phosphatidylcholine (%) | 1 | 6 |
| B | Cholesterol (%) | 0.1 | 1 |
| C | Ethanol (%) | 10 | 45 |

done to determine the average particle size, PDI and stability of the transfersomes and ethosomes. It was analyzed using Malvern Zeta sizer. 1 ml of the sample was suitably diluted up to 10 ml with distilled water. The solution was transferred into the cuvettes and then loaded into the machine. It was examined for its particle size, PDI and zeta potential (Ahmede, S. *et al*., 2015).

**Drug entrapment efficiency**

The entrapment efficiency of adapalene loaded transfersmes and ethosomes were determined by centrifugation method. Aliquots of transfersomes were subjected to cold centrifuge at 6000 rpm for 2 hrs at 4ºC. Aliquots of ethosomes were subjected to cold centrifuge at 10,000 rpm for 1 hr. 1 ml of the supernatant liquid was diluted up to 10ml with phosphate buffer of pH 7.4 and is analyzed by UV-Visible spectrophotometer at 277nm.

The percentage entrapment efficiency of the formulation was calculated using the following formula (Jain, K.S. *et al*., 2008).

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of Drug Recovered}}{\text{Total amount of Durg}} \times 100$$

**Turbidity measurement**

Turbidity of the transferosomal and ethosomal formulation was determined by the Nephelometer. In this method, the 500 NTU were kept then zero reading is kept with the Millipore water solvents. The transfersomal and ethosomal formulation was diluted to obtain 1% solution and was transferred into 50 ml capacity cuvettes and was placed in the holder inside the instrument. Turbidity measurement displayed on the screen was recorded (Sigh, H.P. *et al*., 2009).

**Preparation of adapalene loaded transfersomal and ethosomal gel**

The transfersomal and ethosomal gel containing adapalene were prepared by incorporating formulated vesicles in 1% carbopol 934 gel. 0.5 gm of the carbopol was weighed and was added into 50 ml distilled water and allowed to swell for 24 hrs at room temperature. 50 ml of the prepared formulation was incorporated into the gel and allowed to stir for 20 min. 0.1% of triethanolamine was added and stirred until the transparent gel was obtained. Propylene glycol was added in stirring to obtain the desired consistency. pH was adjusted using 1% triethanolamine solution.

**Evaluation of the gel**

• Measurement of pH

The pH of the gels was determined using a pH meter. The electrode of the electronic pH meter was kept in contact with the surface of the prepared gel and was allowed to equilibrate for 1 min. The pH of gels prepared was determined in triplicate at room temperature (David, S.R. *et al*., 2013).

• Viscosity Measurement

The viscosity of the gels containing transfersomal and ethosomal vesicles was determined using Brookfield viscometer DV- II + pro equipped with spindle no. S-93 at different rpm of 10, 20, 50, 100 rpm at 25 ± 1ºC (Akhtar, N. *et al*., 2012).

• Spreadability

A gel of 0.5 gm was placed between two transparent glass slides on the portion of 1cm premarked on the glass slide and second glass slide was employed. A weight of 100 gm was allowed to rest on the upper glass slide for 5 min. A weight of 50 gm was kept on the balance and the upper slide was allowed to slip off. The time taken by the slides to cover the distance of 6cm was noted (Rai, S., 2017).

Spreadability was calculated by using the following formula:

$$\text{Spreadability} = \frac{m \times l}{t}$$

Where S = spreadability

m = weight tied to the upper slide (0.5 g)

l = length of the glass slide (6 cm)

t = time taken in seconds

**Drug content**

A gel of 0.1 gm equivalent to 100 mg of the drug was taken in 100 ml of phosphate buffer pH 7.4 and stirred on a magnetic stirrer for 24 hrs. The solution was filtered, 1ml of the solution was diluted up to 10 ml with phosphate buffer pH 7.4. It was analyzed spectrometrically by using UV-Visible spectrophotometer (Thakur, N., 2018).

***In vitro* drug release study**

*In vitro* drug release studies was carried out using modified Franz diffusion tube. The receptor compartment was filled with 50 ml of the buffer phosphate buffer pH 7.4 and temperature of dissolution medium was maintained at 37 ± 0.5ºC. 1 gm of the gel was placed on cellophane membrane attached to the tube. The whole set up was dipped in phosphate buffer and was kept stirring using magnetic stirrer at 250 rpm. Three ml of the sample was collected from the receptor compartment at a predetermined time interval and was replaced with the same amount of buffer. 1 ml of the sample was diluted up to 10 ml with phosphate buffer pH 7.4 and absorbance was measured at 277 nm. The same was repeated with the marketed gel.

### *Ex vivo* skin permeation study

The *ex vivo* release studies of the optimized formulations and the gel were carried out using the goatskin membrane. The dissolution medium used was phosphate buffer pH 7.4. The skin was mounted to one end of the glass tube with stratum corneum facing the donor compartment. 1 gm of the gel was placed on membrane and the glass cylinder was attached to the shaft and suspended in 50 ml of dissolution medium. The temperature was maintained at 37ºC and stirred at 250 rpm using magnetic stirrer. 3 ml of the sample were collected at a suitable interval andthe absorbance was measured. The same was repeated with the marketed gel.

### Skin deposition study

At the end of 24 h, the skin mounted on the diffusion cell was removed carefully and washed five times with warm (45ºC) receptor medium. The skin was cut into small pieces and was washed completely in a beaker. To it 10 ml of 50% ethanol was added was mechanically shaken at 37ºC for 2 hr for the complete extraction of the drug. After suitable dilution, it was analyzed by UV spectroscopy at 277 nm (Iizhar, S.A., 2016).

### Skin irritation study

The protocol for the present study (Ref: NGSM/IAEC/2018-19/85) was approved by the Institutional Animal Ethics Committee (IAEC). Skin irritation study was carried out using albino rats for product safety as per OECD guideline 404 "acute dermal irritation/corrosion". The skin irritation study was performed on 12 healthy albino rats weighing between 150-200 gm of either sex. The selected rats were divided into 4 groups (Group I, group II, group III, group IV) and caged separately according to the group. The study animals were provided with normal food, water and environmental conditions. The hair on the dorsal portion of the skin was shaved without damaging the skin, 24 hr prior to the test. Blank gel, marketed gel,transfersomal gel and the ethosomal gel was applied on the shaved area and covered by a gauze patch held using a bandage. After 24 hrs the patch was removed and the skin was cleaned using gauze after soaking in warm water. The responses were determined as per OECD guidelines at 1, 24, 48 and 72 hr.

### Stability studies for transfersome and ethosomes

The stability studies were carried out as per ICH guidelines for 3 months. The study was carried out at 25ºC ± 2ºC and 40 ± 0.2% RH.

Transfersomal and Ethosomal gel was placed in the air tight glass container at a clean and dry place in cool temperature and at room temperature for three months. After the specific time interval, the gels were evaluated for particle size, zeta potential, pH, viscosity and drug content.

## Results and Discussion

### Optimization of transfersomes and ethosomes by design expert software

The results of the particle size, PDI, zeta potential and entrapment efficiency of adapalene loaded transfersomes and ethosomes are shown in table 3. The results indicate that the extent of adapalene incorporation was significantly influenced by all the variables studied for transfersomes and ethosomes. The 30 experimental batches were examined for transfersomes and 20 experimental batches for ethosomes. As per the results obtained, particle size was found to be in between 118.33 nm to 793.3 nm for transferosomes and 101.1 nm to 408.6 nm for ethosomes. PDI was found to be in the range of 0.294 to 1.00 for transferosomes and 0.335 to 0.739 for ethosomes. Zeta potential of the formulation was found to be between -67.6 to -26.7 mV for transferosome and -77.6 to -36.8mV for ethosome. The entrapment efficiency was found to be in the range of 12.4% to 73.5% for transferosomes and 66.44% to 96% for ethosomes.

The obtained polynomial equations given below were used to draw the conclusions based on the magnitude of the coefficient, as well as the sign (+or –) associated with it. The estimated coefficient value of particle size, PDI, zeta potential, entrapment efficiency was found to be statistically significant with p-value <0.05. These observations conclude that this was the best representative model for the study. The positive sign associatedwith coefficients A, B, C and D indicated a positive correlation between the studied variables and the obtained responses for particle size, PDI, zeta potential and entrapment efficiency. The influence of the studied variables on the responses is also shown in the form of the coded equation and contour plot.

The mathematical model containing coefficient effects, interactions and polynomial terms was analyzed to assess the response using the following equations:

### For transfersomes

- The equation for particle size:

Particle size = + 397.55 - 68.29*A - 4.33*B + 40.04*C

**Table 3:** The viscosity of the optimized transfersomal and ethosomal gel.

| Shear stress (rpm) | Viscosity in cps | |
|---|---|---|
| | Transfersomal gel | Ethosomal gel |
| 10 | 568.96 | 552.66 |
| 20 | 274.33 | 218.8 |
| 50 | 108.83 | 108.66 |
| 100 | 59 | 56.13 |

-39.50*D - 44.99*AB 34.16*AC + 12.48*AD -47.17*BC -11.29*BD + 8.21*CD -15.65*$A^2$ - 53.005*$B^2$ - 46.59*$C^2$ + 24.49*$D^2$

Where, A, B, C and D represent the coded values for soya lecithin, cholesterol, Tween 80 and sodium deoxycholate respectively.

• The equation for PDI:

PDI = +0.7973 – 0.1322*A - 0.0289*B + 0.0305*C -0.0277*D - 0.0101*AB - 0.0715*AC + 0.0400*AD - 0.0446*BC - 0.0106*BD - 0.0227*CD - 0.0694*$A^2$ - 0.0295*$B^2$ - 0.0745*$C^2$ + 0.0149*$D^2$

Where, A, B, C and D represent the coded values for soya lecithin, cholesterol, Tween 80 and sodium deoxycholate respectively.

• The equation for zeta potential :

Zeta potential = -44.29.

• The equation for entrapment efficiency:

Entrapment efficiency = + 49.42 + 6.78*A - 0.9467*B + 2.44*C + 3.26*D

Where, A, B, C and D represent the coded values for soya lecithin, cholesterol, Tween 80 and sodium deoxycholate respectively.

**For ethosomes**

• The equation for particle size:

Particle size = + 191.38 – 54.77*A + 22.61*B – 34.86*C

Where, A, B and C represent the coded values for soya phosphatidylcholine, cholesterol and ethanol respectively.

• The equation for PDI:

PDI = + 0.5615 - 0.0884*A + 0.0441*B - 0.02268*C

Where, A, B and C represent the coded values for soya phosphatidyl choline, cholesterol and ethanol respectively.

• The equation for zeta potential:

Zeta potential = -49.54 + 2.56*A + 0.1917*C - 5.16*C + 7.84*AB + 9.56*AC - 5.34*BC

Where, A, B and C represent the coded values for soya phosphatidyl choline, cholesterol and ethanol respectively.

• The equation for entrapment efficiency:

Entrapment efficiency= + 71.48 + 1.49*A - 0.9847*B + 3.38*C - 0.4675*AB + 5.49*AC - 0.6975*BC + 5.10*$A^2$ +3.73*$B^2$ - 0.8012*$C^2$

Where, A, B and C represent the coded values for soya phosphatidyl choline, cholesterol and ethanol respectively.

The response surface graph for particle size, PDI and zeta potential of transfer some indicate that as the concentration of soya lecithin and cholesterol increases, there was a decrease in particle size, PDI and zeta potential. Response surface graph for entrapment efficiency indicates that as the concentration of soya lecithin and cholesterol increase, entrapment efficiency was found to increase.

The response surface graph for particle size, PDI and zeta potential of ethosome indicate that as the concentration of soya phosphatidylcholine and cholesterol increases, there was a decrease in particle size, PDI and zeta potential. Response surface graph for entrapment efficiency indicates that as the concentration of soya phosphatidylcholine and cholesterol increase, entrapment efficiency was found to increase.

The following figures represent the response surface graphs obtained for optimization of the vesicles.

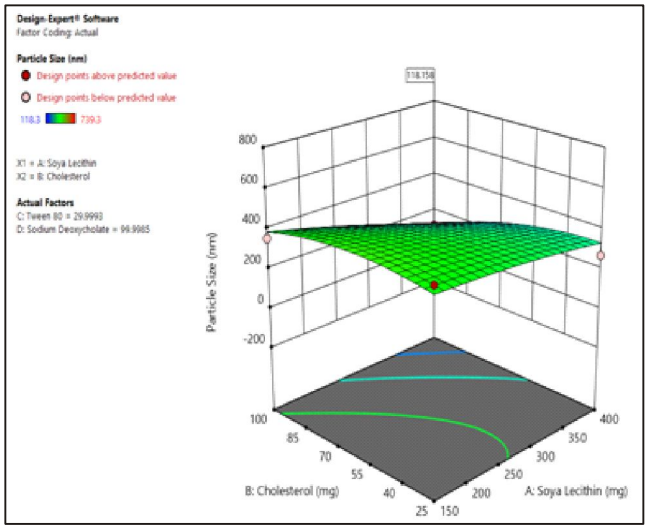


**Fig. 1:** Counterplot for the particle size of transfersomes.

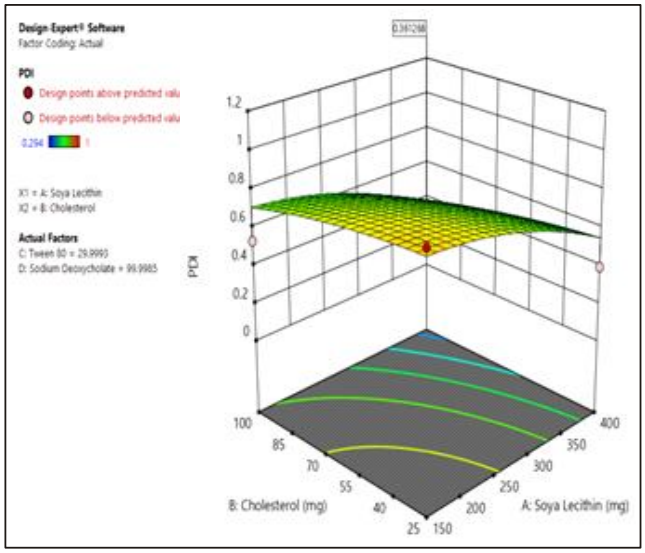


**Fig. 2:** Counter plot for PDI of transfersomes.

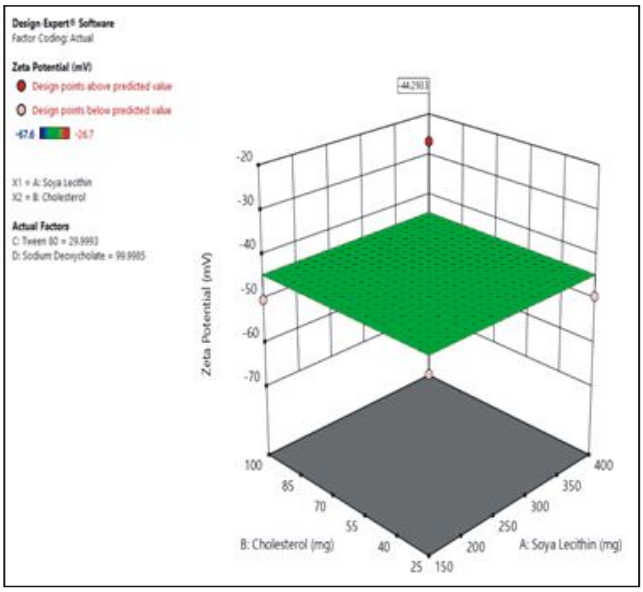


**Fig. 3:** Counterplot for zeta potential of transfersomes.

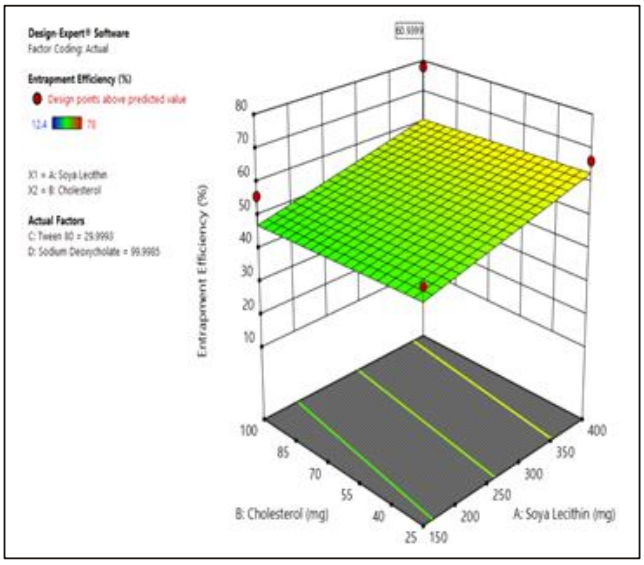


**Fig. 4.** Counterplot for entrapment efficiency of transfersomes.

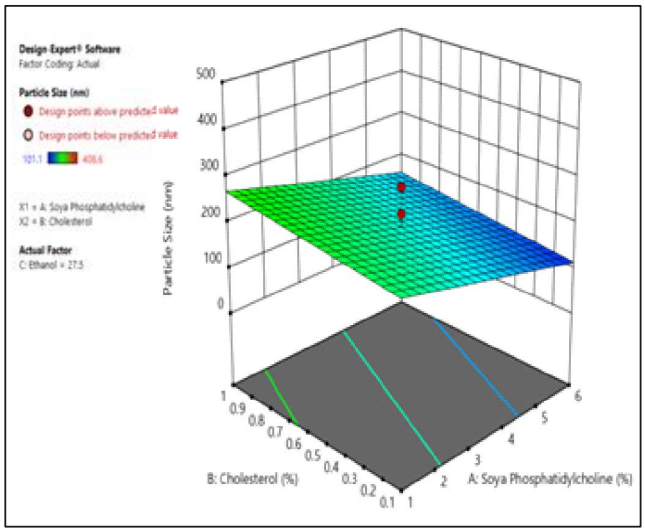


**Fig. 5:** Counterplot for the particle size of ethosomes.

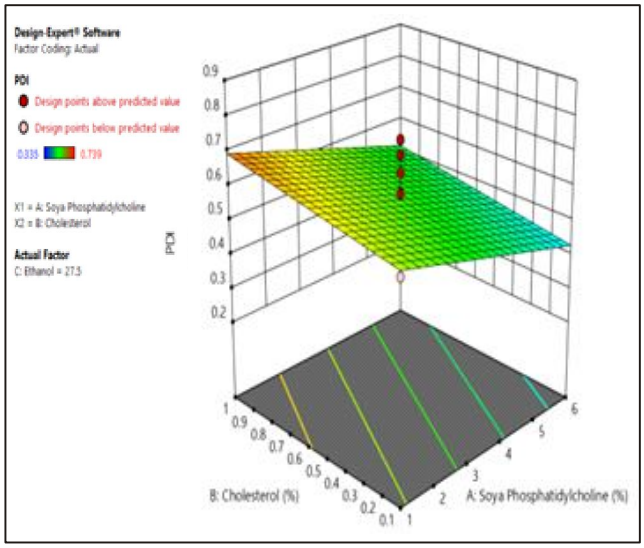


**Fig. 6:** Counterplot for PDI of ethosomes.

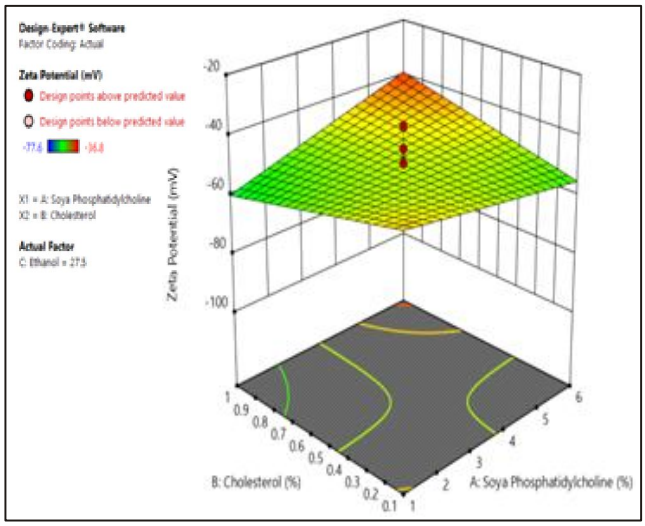


**Fig. 7:** Counterplot for zeta potential of ethosomes.

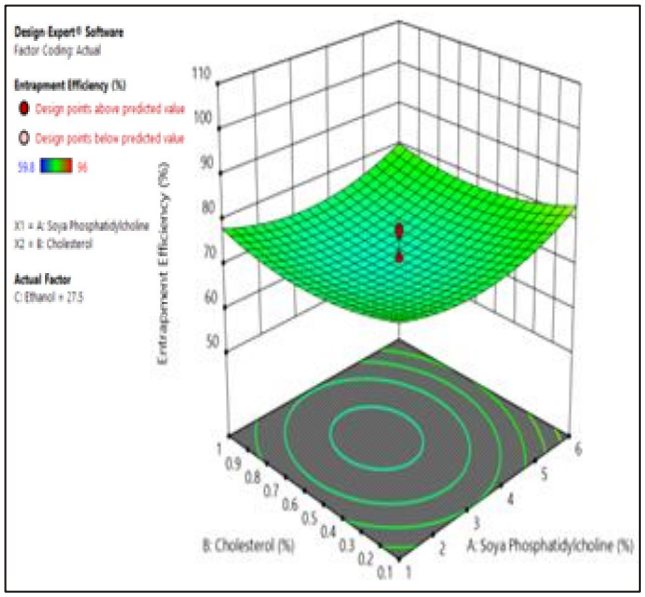


**Fig. 8:** Counterplot for entrapment efficiency of ethosomes.

### Turbidity

Nephelometer was used to find the turbidity of the optimized formulations. The value obtained was 230 for optimized transfersomes and 380 for optimized ethosomes. Greater the turbidity, higher the number of vesicles present in the system. Hence the values indicate that the maximum number of vesicles were present in the system.

### Measurement of pH

The pH of the gels was determined using a pH meter. The pH of the optimized transfersomal gel was found to be 6.81 ± 0.01 and that of the optimized ethosomal gel was found to be 6.1 ± 0.01. The pH of the gel was found to be in the range of 6.1-6.8 which is closer to skin pH. Hence it indicates that the chances of irritation were less.

### Viscosity

The results obtained for the viscosity of the optimized gels are tabulated in the tabular column below. (Table 3) From the graph obtained we can conclude that as the shear rate increases, viscosity decreases and vice versa.

### Spreadability

The spreadability of the optimized transfersomal gel was found to be 23.026 ± 3.178 g/cm$^2$. The spreadability of the optimized ethosomal gel was found to be 23.58 ± 1.74 g/cm$^2$. The spreadability of the gel plays an important role in the application, if spreadability is poor then ultimately it hampers the drug residence time on the skin which may lead to poor bioavailability. Based on the values obtained, spreadability was found to be good.

### Drug content

The drug content of the optimized transfersomal gel

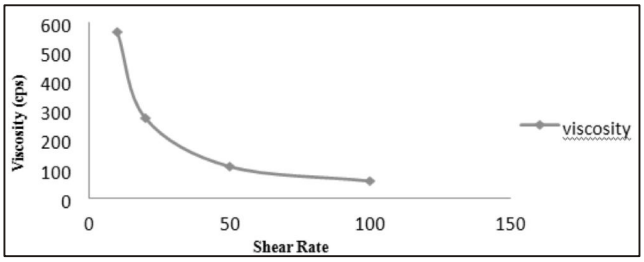


**Fig. 9:** The viscosity of optimized gel containing transferosome.

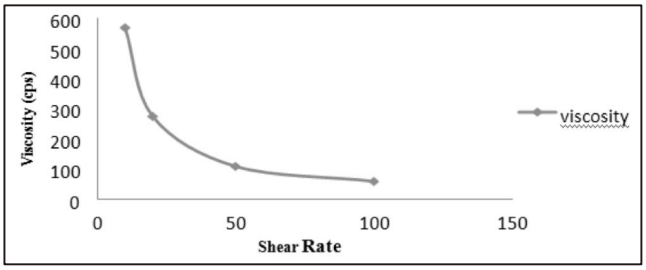


**Fig. 10:** The viscosity of optimized gel containing ethosome.

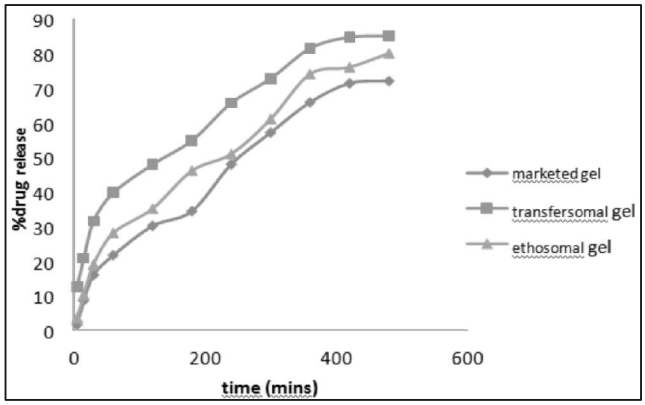


**Fig. 11:** Comparison of *in vitro* release studies of transferosomal, ethosomal and marketed gel.

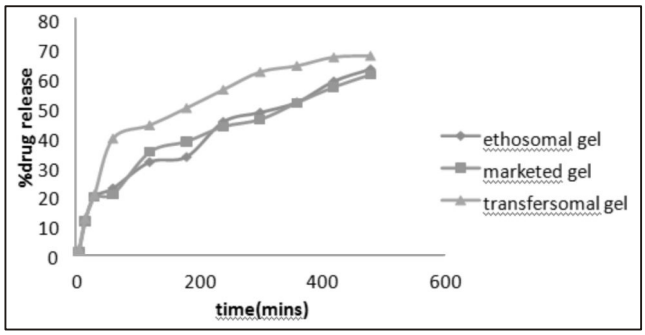


**Fig. 12:** Comparison of *ex vivo* release studies of transferosomal, ethosomal and marketed gel.

was found to be 89.23% and 87.73% for optimized ethosomal gel. The drug content determination also showed that the drug was uniformly distributed throughout. Drug content uniformity is essential for semi-solid preparation to confirm the homogeneity of the dispersed drug throughout the formulation.

### *In vitro* drug release

*In vitro* studies showed that the % cumulative drug release from the optimized transfersomal gel, ethosomal

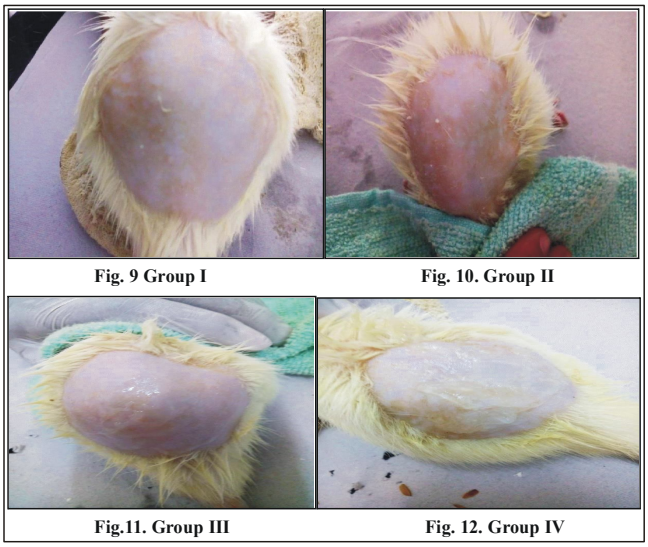


**Fig. 9-12:** Skin irritation studies for the optimized gel.

**Table 4:** Data for *in vitro* drug release.

| Time (mins) | % Cumulative release | | |
|---|---|---|---|
| | Transfersomal gel | Ethosomal gel | Marketed gel |
| 5 | 12.52 | 3.45 | 1.44 |
| 15 | 20.71 | 10.14 | 8.43 |
| 30 | 31.39 | 18.94 | 15.81 |
| 60 | 39.56 | 27.99 | 21.54 |
| 120 | 47.78 | 35.03 | 30.01 |
| 180 | 54.67 | 45.99 | 34.32 |
| 240 | 55.00 | 50.83 | 47.85 |
| 300 | 72.59 | 60.92 | 56.98 |
| 360 | 81.321 | 73.94 | 65.72 |
| 420 | 84.52 | 75.90 | 71.22 |
| 480 | 84.883 | 79.92 | 71.89 |

gel and marketed adapalene gel was found to be 84.88%, 79.92% and 71.89% respectively. (Table 4). Based on the result obtained for drug release comparisons studies, it was observed that the amount of drug released from the optimized transfersomal gel was found to be in greater than other gels. It was suggested that the spontaneous release of atransfersome with a nano-size permitted the sustained amount of drug release. The cumulative % drug release profile and the graph for the same given below.

### *Ex vivo* skin permeation study

*Ex vivo* permeation studies of the optimized gels were carried out using modified Franz diffusion cell using goat skin membrane. Results of the *ex vivo* permeation studies revealed that there were 67.39%, 62.87% and 61.01% drug release for transfersomal gel, ethosomal gel and market edgel respectively at the end of 8hrs across the skin. The skin permeation profile showed the same pattern as it was shown by *in vitro* release profile across the cellophane membrane. The results also revealed that transfersomal gel showed significantly higher permeation. (Table 5).

**Table 5:** Data for *ex vivo* drug release.

| Time (mins) | % Cumulative release | | |
|---|---|---|---|
| | Transfersomal gel | Ethosomal gel | Marketed gel |
| 5 | 2.09 | 1.95 | 1.23 |
| 15 | 12.47 | 12.00 | 11.48 |
| 30 | 20.47 | 19.84 | 19.78 |
| 60 | 39.48 | 22.65 | 20.58 |
| 120 | 43.94 | 31.52 | 34.89 |
| 180 | 49.75 | 33.26 | 38.45 |
| 240 | 55.93 | 45.10 | 43.45 |
| 300 | 61.93 | 48.21 | 45.87 |
| 360 | 63.98 | 51.69 | 51.51 |
| 420 | 66.92 | 58.74 | 56.82 |
| 480 | 67.39 | 62.87 | 61.01 |

**Table 6:** Kinetic models of optimised formulation.

| Formulation | Model | R2 | K | N |
|---|---|---|---|---|
| Transfersomal gel | Zero- order | 0.966 | -0.152 | - |
| | First- order | 0.700 | 0.002 | - |
| | Higuchi | 0.974 | 0.666 | - |
| | Korsmeyerpeppas | 0.974 | 0.138 | 0.666x |
| Ethosomal gel | Zero- order | 0.967 | -0.147 | - |
| | First- order | 0.650 | 0.002 | - |
| | Higuchi | 0.983 | 3.651 | - |
| | Korsmeyer-Peppas | 0.945 | -0.113 | -0.759 |

### Release Kinetics

The data obtained from the *in vitro* drug release studies were fitted to zero order, first order, Higuchi matrix and Korsmeyer-Peppas model. The results obtained were given in (Table 6).

### Skin deposition

Skin deposition study was conducted and transfersomal gel showed skin retention values of 78.84% and 75.96% for ethosomal gel. The transfersomal gel was found to have better entrapment efficiency and skin deposition compare to ethosomal gel. This shows that transfersomal gel exhibited a greater amount of drug deposition in the skin membrane and it acts as a depot and releases the drug in a sustained manner.

### Skin irritation studies

After inspecting visually, the prepared transfersomal gel and the ethosomal gel was found to be compatible with rat skin and showed no irritation (Table 7 ). The rats were observed after 0 hr, 24 hr, 72 hr and the readings were noted. There were no signs of any erythema formation in the entire period of 72 hr with adapalene loaded transfersomalgel but whereas the group II and group III showed an irritation in terms of color change in skin and redness and increased. As suggested in the results, the side effect of the marketed gel is overcome by the transfersomal gel. From the results, it can be concluded that adapalene loaded transfersomal gel was found to be irritation free but the marketed formulation

**Table 7:** The score of skin irritation.

| Skin response | Type | Parameters | | Primary irritation index |
|---|---|---|---|---|
| | | Erythema | Edema | |
| Group 1 | Test | 0 | 0 | 0 |
| | Control | 0 | 0 | 0 |
| Group 2 | Test | 1 | 0 | 0.5 |
| | Control | 0 | 0 | 0 |
| Group 3 | Test | 0 | 0 | 0 |
| | Control | 0 | 0 | 0 |
| Group 4 | Test | 1 | 0 | 0.5 |
| | Control | 0 | 0 | 0 |

**Table 8:** Stability study of the optimized formulation at 25ºC ± 2ºC and 40 ± 2% RH.

| Formulation | parameters | Time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 |
| Transfersome | Particle size (nm) | 118.3 | 118.3 | 145.0 | 152.1 |
| | PDI | 0.398 | 0.398 | 0.447 | 0.533 |
| | Zeta potential (mV) | -44.2 | -44.2 | -40.5 | - |
| | Entrapmentefficiency (%) | 68.33% | 68.01% | 67.37% | 65.43% |
| Ethosome | Particle size (nm) | 192.3 | 192.3 | 208.2 | 262.3 |
| | PDI | 0.523 | 0.523 | 0.627 | 0.788 |
| | Zeta potential (mV) | -49.5 | -49.5 | -45.4 | -44.2 |
| | Entrapmentefficiency (%) | 63.44% | 63.23% | 62.88% | 60.94 |

and ethosomal gel showed slight irritation.

**Stability studies for transfersomes and ethosomes**

Accelerated stability studies of the optimized formulations were conducted for 3 months and the results obtained are given in the (Table 8).

## Conclusion

The present study highlights the strategy of comparing the efficiency of two types of vesicular system, i.e. transfersome and ethosome. Adapalene loaded transfersome was successfully formulated by using the reverse phase evaporation method. Ethosome containing adapalene were prepared using the cold method. The formulations were later incorporated into carbopol gel. Adapalene is the third generation naphthoic acid derivative with retinoid activity. Vesicular systems are used so as to overcome the disadvantages such as lower bioavailability, low permeability, drug release and stability problems associated with the drug. Stratum corneum acts as a protective barrier to the skin and thereby prevent the particles from penetrating through it. Hence these lipid vesicular systems i.e. transfersomes and ethosomes were used to overcome the above-mentioned disadvantages.

The formulations were optimized using DoE software. The factors used for the optimization of transfersome were soya lecithin, cholesterol, tween 80 and sodium deoxycholate. Soya phosphatidylcholine, cholesterol, ethanol were the factors considered for ethosomes. The responses for optimization of the vesicles were particle size, PDI, zeta potential and entrapment efficiency. The *in vitro drug* release study revealed that the transfersomal gel exhibited more prolonged drug release as compared to ethosomal gel and marketed gel of Adapalene (0.1%w/w). The *ex vivo* drug release was found to be slow as compared to the *in vitro*, release studies due to much time required for the drug to diffuse through the goatskin membrane. Also, the transfersomes showed a greater sustained release when compared to ethosomal and marketed gel. The skin irritation studies on albino rats showed no particular reaction or irritation on the application of the transfersomal gel, whereas ethosomal gel and the marketed gel showed slight irritation and color change of the skin. Based on the results obtained, adapalene loaded transfersomal gel was found to be better as compared to that of ethosomal gel for the treatment of acne.

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## Conflict of Interest

Authors declare no conflict of interest

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