How to Cite:
https://doi.org/10.53730/ijhs.v6nS6.10118

**Design and development of tofacitinib citrate loaded transfersomal gel for skin cancer by box-Behnken design- doe approach**

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**Abstract**---Objective: The purpose of the present study is to develop, statistically optimize and evaluate the transfersomal gel formulation of Tofacitinib citrate via transdermal route by DOE Approach. Materials and Methods: Transferosomes are made up of with bilayer forming phospholipids and biocompatible surfactants like sodium deoxycholate. The surfactants also acts as edge activator which stabilizes the phospholipid bilayer and increases the deformability of the vesicle. The formulation were designed by Box-Behnken Design. For usage as a transfersomal gel, drug encapsulation in various transfersomal formulations having 100 mg drug concentrations and Carbopol-934 (0.5,1.2 g) is being studied. Results: Entrapment efficiency (EE percent), drug content, in-vitro skin penetration testing, and stability studies were all found in the produced formulations. Transmission Electron Microscopy confirmed that the vesicles were spherical in shape. According to the findings, Tofacitinib citrate was effectively pinned with a standardised drug concentration in all formulations. The 0.1 g Tofacitinib citrate optimised transfersome formulation TG2 exhibited encouraging results, with maximum drug release (94.32%). Conclusion: Transferosomes are a promising long-term delivery route for Tofacitinib citrate and are relatively stable. This research work reveals that transfersomes containing Tofacitinib citrate could be used to treat squamous cell carcinoma via transdermal drug delivery.

**Keywords**---transfersome, edge activator, felixibility, penetration, tofacitinib citrate, skin cancer, permeation.
Introduction

Skin cancer was projected to account for more than a third of all cancers over two decades ago. According to World Health Organization (WHO) approximately 65,000 people globally die from melanoma every year. According to the American Cancer Society, over 91,270 new instances of melanoma will be detected in the United States in 2021, with 9,320 people dying from the disease. Surgery, chemotherapeutics, radiotherapy, biological therapy, and targeted therapy are currently used to treat melanoma. Surgical removal is restricted to early-stage melanoma, and the use of a chemotherapeutic drug or combination therapy was associated with severe adverse effects, poor response, and survival rate. The most frequent type of skin cancer in the first category is basal cell carcinoma (BCC). BCC seldom spreads beyond the primary tumor location and rarely becomes fatal. It can, however, be disfiguring if not treated promptly. The majority of severe or malignant melanomas are dark-colored pigmented lesions. Although the majority of instances are treatable, they can result in mortality. MSC skin cancer is the worst form of skin cancer. Damaged DNA generates mutations in melanoma, which are genetic faults that allow tumoral skin cells to reproduce rapidly.

Gregor Cevc proposed the concept of transfersomes in 1992. They are also known by different names such as ultradeformable liposomes, flexible liposomes, elastic liposomes, and deformable liposomes. Transfersomes are made up of with bilayer forming phospholipids and biocompatible surfactants like sodium cholate, sodium deoxy cholate, tweens, spans, and dipotassium glycyrrhizinate. The surfactant acts as an edge activator, destabilizing the phospholipid bilayer and increasing the vesicle’s deformability/elasticity. Due to their elasticity nature, they are capable to squeeze through the narrow pores about 5-10 times lesser than their specific diameter. Many tests must be carried out after the formulation approach has been developed in order to develop a final formulation. The application of a systematic methodology and DOE has proven to be a very effective technique for formulation development. DOE enables the formulation scientist to assess various components and their interactions while maintaining complete control over the number of experiments. We utilized Design-Expert to create a 3 level 3 factorial design Box-Behnken design (Version 12, Stat-Ease Inc., Minneapolis, MN).

Materials and Methods

The pure drug Tofacitinib was obtained as a gift sample from Pfizer Inc. soya lecithin, methanol, and chloroform were procured from Delpha Drugs and Pharmaceuticals India. S.D. Fine Chemicals Ltd., India, soya phosphatidyl choline, Carbopol-934, isopropyl alcohol, and potassium dihydrogen orthophosphate. All of the chemicals used in the experiments were of analytical grade. Purified water that had been freshly prepared was used. The formulations were done by using Box-Behnken design design expert software version 12.
Experimental design for optimization of drug loaded transfersomes using factorial design

The primary purpose of this research was to apply a mathematical model based on the Box-Behnken design to discover the best circumstances for producing transfersomes with desired properties. To characterize the relationship between independent and dependent variables, as well as their responses, the response surface technique was combined with the Box–Behnken design. A three-factor and three-level design with three replicates at the center was chosen to generate response surface models. The chart depicts statistically significant variables on the parameters under consideration.

Preformulation studies
Compatibility studies through FT-IR

FTIR spectra obtained through the compatibility of the pure drug and excipient was observed using Bruker FTIR. Drug compatibility with phosphatidyl choline (PC), sodium deoxycholate and carbopol was studied. The FTIR technique is used to investigate the various functional groups of guest and host molecules by examining significant changes in the shape and position of absorbance bands. The data from the FTIR Spectrophotometric analysis clearly reveals that the spectra obtained from the physical mixing of Tofacitinib and excipients vary significantly. The spectrum of mixtures of tofacitinib with both the excipients (phosphotidylcholine, sodium deoxycholate and carbopol) respectively showed all the characteristic peaks of tofacitinib at 3550 cm⁻¹ (O-H), 1750 cm⁻¹ (C≡O), 3000 cm⁻¹ (C-H stretching), 1600-1300 cm⁻¹ (C-C stretching vibration) and 743-739 cm⁻¹ (C-O-C stretching vibration with slight variation or shifting in the peaks). However, no additional peaks other than peaks of individual components were observed. Thus indicates compatibility of tofacitinib with selected excipients. The results have been shown in the Figure 1. Graphical representation is as shown in the figure 2.

Figure 1. Overlap of FTIR OF Drug and excipients
Standard calibration curve of Tofacitinib citrate

![Calibration curve of Tofacitinib](image)

Figure 2. calibration curve of Tofacitinib citrate

**Optimization of Formulation of Tofacitinib loaded transferosomes**

**Preparation of transferosomes**

Phosphatydylcholine, sodium deoxycholate, and the Tofacitinib citrate were dissolved in 10 mL of a mixture of two organic solvents (chloroform: methanol) at suitable ratio as shown in the formulation table 1 in a clean, round bottomed flask. A magnetic stirrer was used to carefully evaporate the organic solvent to form a lipid layer on the flask wall, and a phosphate buffer solution (pH 7.4) was hydrated by rotating at room temperature at 60 rpm for 1 hour and then held at room temperature for 2 hours for swelling. The multilaminar lipid vesicles (MLV) are then sonicated for 10 minutes with a probe sonicator (Heldolph vcx750) as shown in the table 1.

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>LEVELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable</td>
<td>Low</td>
</tr>
<tr>
<td>$X_1$=Phosphatydylcholine(mg)</td>
<td>30</td>
</tr>
<tr>
<td>$X_2$= Sodium deoxycholate(mg)</td>
<td>20</td>
</tr>
<tr>
<td>$X_3$=Solvent mixture(Choloform:methanol) ml</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1$= Particle size(nm)</td>
</tr>
<tr>
<td>$Y_2$=Entrapment efficiency %</td>
</tr>
<tr>
<td>$Y_3$=Polydispersivity index PDI</td>
</tr>
</tbody>
</table>
Evaluation Parameters
For transfersomes
Vesicular size determination

Photon correlation spectroscopy is used to assess the diameter of the vesicle. Distilled water is used to prepare a sample. After passing through the 0.2 mm membrane filter, the samples are diluted with filtered saline.\textsuperscript{11}

Zeta Potential Analysis

The optimized formulation’s zeta potential, size distribution, and vesicle size were determined using Zetasizer (DTS Version 5.03, Malvern) and the light scattering technique, also known as photon correlation spectroscopy (PCS). In this setup, the zeta sizer is adjusted to 25°C at a 90° angle. Water is utilized as a surfactant in the calculation of Zeta potential and size determination.\textsuperscript{12}

Entrapment efficiency

Entrapment efficiency is stated as a percentage of the drug concentration added. To separate the confined drug, mini-column centrifugation was used.\textsuperscript{13}

\[
\text{Entrapment efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100
\]

Percentage Drug Content

A transferosome formulation of approximately 1gm was used to determine the percentage drug content. Sonication with ethanol was used for 15 minutes to lyse the vesicles. Centrifugation at 14000 rpm was performed for half an hour by placing the solution in a centrifugation tube. To dilute the clear solution obtained, 100 ml of methanol was employed. Diluting 10 ml of the produced solution yielded 100 ml of pH 7.4 phosphate buffer. Tofacitinib citrate drug concentration was determined using a UV spectrophotometer at 285 nm after aliquots were withdrawn at regular time intervals.\textsuperscript{14}

\textbf{In-Vitro Drug Release Studies}

A cellophane membrane (Molecular weight cut off 12000-14000, HI Media Ltd, Mumbai, India) was used to test alternative transfersomal formulation drug release statistics. A precise amount of formulation was spread out across a membrane with an accessible diffusion zone located between the donor and receptor chambers (Franz-diffusion cell apparatus). The receptor compartment is filled with a constantly stirred phosphate buffer pH 7.4 at a rate of 50 rpm with a tiny magnetic bar at a temperature of 37.5 °C. 5 ml aliquots were extracted and reconstituted with the same amount of phosphate buffer solution at various time intervals. To establish the invitro drug release model, the samples were examined in a spectrophotometer and a graph illustrating the amount of drug entering through the membrane over time was created as shown in the table 2.\textsuperscript{15}
### Table 2
Optimization table 3 level 3 factorial design of transfersomes

<table>
<thead>
<tr>
<th>Number of formulations</th>
<th>Phosphatidylcholine(mg)</th>
<th>Sodium deoxycholate(mg)</th>
<th>Solvent mixture</th>
<th>Particle size(nm)</th>
<th>Entrapment efficiency(%)</th>
<th>Polydispersivity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>40</td>
<td>2</td>
<td>118.3</td>
<td>84.04</td>
<td>0.245</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>40</td>
<td>2</td>
<td>119.2</td>
<td>89.03</td>
<td>0.258</td>
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<tr>
<td>3</td>
<td>60</td>
<td>40</td>
<td>2</td>
<td>118.8</td>
<td>87.09</td>
<td>0.245</td>
</tr>
<tr>
<td>4</td>
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<td>119.4</td>
<td>88.64</td>
<td>0.265</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>40</td>
<td>2</td>
<td>119.7</td>
<td>89.46</td>
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<td>0.884</td>
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<td>309</td>
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<tr>
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<td>3</td>
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<td>223</td>
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<td>0.265</td>
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<td>60</td>
<td>40</td>
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<td>0.976</td>
</tr>
<tr>
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<td>60</td>
<td>40</td>
<td>2</td>
<td>119.7</td>
<td>83.35</td>
<td>0.265</td>
</tr>
</tbody>
</table>

**Effect of phospholipid: surfactant ratio on Entrapment Efficiency and Drug Loading**

The % entrapment efficiency of deformable vesicle formulations ranged from 28.970.03 to 89.840.34 (Table 1). With increasing surfactant concentration from 5 to 10% (w/w) in transfersomes produced with sodium deoxycholate, the percent EE increased significantly (P<0.05). When surfactant concentrations above 15%, mixed micelles coexisted with transfersomes, resulting in lower drug entrapment due to the stiffness and smaller size of mixed micelles. According to Patel et al., the influence of phospholipids and surfactant ratio in lipid components of vesicles on the entrapment efficiency of the lipophilic pharmaceutical, Tofacitinib, reduced with increasing surfactant ratio. The phospholipid and surfactant ratios also have an impact on drug loading. The current study discovered that phospholipid and
surfactant concentrations improved drug loading only in a few batches, and overall dependency is modest, but surfactant has a considerable positive effect. The F-value of 15.76 for the model indicates that it is significant. An F-value this large might arise owing to noise only 0.07 percent of the time. Model terms are significant when "Prob > F" is less than 0.0500. as shown in the figure 3& 5.

**Effect of vesicle composition**

The percentages of drug entrapment in various transfersomal compositions are compared to a blank gel and a marketed product in Table 1. The EE of transfersome formulations was significantly higher than that of pure drug suspension and marketed formulation (P>0.05). This result may be due to interactions between the surfactants and tofacitinib, which created a complex that was injected into the transfersomes bilayer. In comparison to liposomes, Fang et al. discovered that adding the surfactant sodium deoxycholate to phosphatidylethanolamine vesicles significantly increased the entrapment efficacy of 5-aminolevulinic acid. Gupta et al. discovered that transfersomes were substantially more successful in trapping than liposomes and niosomes16.

**Effect of phospholipid & surfactant ratio on the Particle Size and PDI**

The ratios of phospholipids and surfactants used in the formulation preparation resulted in a significant change in the average particle size and stability of the transfer group formulation. The phospholipid/surfactant ratio varies greatly, ranging from 85.5 to 95.15 percent. The particle size grows dramatically as the phospholipid/surfactant ratio increases as shown in the figure 4

**Vesicles Size and PDI**

Malvern Mastersizer determined the vesicle size analysis values for transfersomes. The particle size of the formulations F1 to F27 appears to be 118.3 nm to 546 nm when the lipid ratio of phospholipids and surfactant concentration is reduced. Following formulation optimization, the F2 formulation was revealed to have the ideal concentration. As a result, at this concentration, the transfersomal gel must be prepared using the following approach as shown in the figure 4.
Figure 3. Response surface plots particle size

Figure 4. Response surface plots polydispersivity index
Preparation of tofacitinib citrate loaded transferosomal gel using carbopol-934

Carbopol-934 resin in three concentrations. Table 2 was used to determine the appropriate percentage of carbopol-934. The mixture was swirled until it thickened. PEG-400 (5ml) was progressively added into the aqueous dispersion of Carbopol-934 after complete dispersion. Then, 5ml of isopropyl alcohol (IPA), 5ml of propylene glycol (PG), and 1ml of triethanolamine (TEA) were added. To achieve a homogenous gel dispersion, 100g of distilled water (q.s.) was also added. To obtain the best batch of Carbopol-934 gel, these three distinct gel formulations were examined for various assessment parameters. 17-19 Tofacitinib citrate, corresponding to 100 mg medication, was added to the prepared carbopol gel transferosomes, as described in table 4.
Table 4
Formulation table of Tofacitinib loaded Transferosomal Gel

<table>
<thead>
<tr>
<th>S.N O</th>
<th>Transfersome (mg)</th>
<th>Carbopol-934 (mg)</th>
<th>Triethanolamine (ml)</th>
<th>PropyleneGlycol (ml)</th>
<th>IsopropylAlcohol (ml)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFG 1</td>
<td>100</td>
<td>250</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>Q.S</td>
</tr>
<tr>
<td>TFG 2</td>
<td>100</td>
<td>500</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>Q.S</td>
</tr>
<tr>
<td>TFG 3</td>
<td>100</td>
<td>1000</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>Q.S</td>
</tr>
</tbody>
</table>

Transferosomal gel loaded with tofacitinib citrate

**Homogeneity**

Three distinct Carbopol-934 gel compositions were produced and tested for physical appearance using visual inspection. \(^{20}\).

**pH Value of Topical Transferosome Gel**

Each gel formulation’s pH was measured using a digital pH meter to determine its pH. The pH measurement of each system was repeated three times. \(^{21}\).

**Grittiness**

Using a light microscope, the presence of particles in all of the formulations was determined microscopically. The gel formulation meets the need for grittiness and specific matter independence, which is a desirable feature quality for any topical application.\(^ {22}\).

**Spreadability Test**

The gel formulation of 350 mg was placed on one glass slide, and another glass slide with roughly 5.8 g of gel was dropped from a 5 cm distance. The spread gel was checked after 1 minute to estimate the diameter of the circle.\(^ {23}\).

**Extrudability Test**

The gel quantity (g/cm2) extruded from the lacquered aluminum collapsible tube was determined after applying the weight in grams required to extrude at least 0.5 centimeter ribbon of gel in 10 seconds. The extrudability can be calculated using the formula provided. \(^ {24}\).

\[
Extrudability = \frac{\text{Weight applied to extrude gel from the tube (g)}}{\text{Area in cm}^2}
\]
Viscosity

The viscosity of the transferosomal gel containing Tofacitinib was measured using a Brookfield viscometer\textsuperscript{25}.

Transmission and scanning Electron Microscopy Studies

The formed gel was determined using transmission electron microscopy\textsuperscript{26}. Scanning electron microscopic examinations are used to learn about surface morphology \textsuperscript{27}.

Drug Content

1gm of a transferosome gel formulation was used, and the vesicles were lysed by sonication in 25 ml of ethanol for 15 minutes. This solution was then placed in a centrifuge tube and spun at 14000 rpm for 30 minutes. Methanol was used to dilute the clear solution to 100 mL. The solution was then diluted to 100 mL with phosphate buffer pH 7.4. Tofacitinib citrate drug concentration was determined using a UV spectrophotometer at 285 nm from aliquots\textsuperscript{28}.

In-vitro Release Study

The invitro drug release study was carried out in the same manner as the transferosome examination.\textsuperscript{29}

Ex-Vivo permeation study

A Franz Diffusion cell with an efficient diffusion surface area and receiver chamber capacity was used to perform in-vitro permeation on excised, defatted goat skin tissues. The tissue was kept in a deep freezer at \(-21^\circ\) C. It was brought to room temperature before being placed between the donor and receiver compartments of the Franz diffusion cell during the experiment. The donor compartment was facing the goat’s skin, while the receiver compartment was facing the other side. Before the experiment, the goat tissue was stabilized using stimulated skin fluid (SVF) (pH-4.2). SVF was fed into the incubator shaker’s receiver chamber and agitated with a magnetic rotor at a speed of 100 rpm to keep the temperature at 37.1\(^\circ\)C.\textsuperscript{30} To maintain stability, the entire media was reloaded every 30 minutes with new buffer. Following six cycles of stabilisation, 1 mL of the sample (0.5 percent w/v Tofacitinib transferosomal gel) was deposited in the donor compartment with 0.75 mL of SVF to mimic the goat skin milieu condition. The receptor compartment was filled with phosphate buffer (20 mL) (pH 4.5). The samples were collected at predetermined intervals (0.5,1,2,3,4,6,8,10,12,14,16, and 24 hours) and filtered through a 0.45 mm membrane filter\textsuperscript{31}. After that, the samples were tested for drug content using UV spectrophotometry, and the cumulative percentage drug release was computed. The following formulas were used to calculate the flow (mg/cm\(^2\)/h) and permeability coefficient (Kp):

\[
\text{Flux (mcg/cm}^2/\text{h)} = \text{Cumulative amount of drug permeated vs time}
\]

\[
\text{Permeability coefficient (Kp)} = \frac{\text{Flux}}{\text{Drug concentration in donor compartment}}
\]
Stability Study

For the stability research, the formulation was kept at room temperature (25 ± 2°C) for two months. pH, spreadability, and extrudability were tested after the first and second months to evaluate the formulation.

Results and Discussion

Preformulation studies

Drug Excipient Compatibility Study by FT-IR

The overlap figure of tofacitinib citrate + sodium deoxycholate + phosphatidylcholine and carbopol 934 seems to be significant. It is observed that there were no compatibilities between drug and excipients.

Figure 6. Overlap figure of FTIR of 1. tofacitinib (TFG1), 2. tofacitinib with sodium deoxycholate and phosphatidylcholine (TFG2) 3. Tofacitinib with sodium deoxycholate, phosphatidylcholine and carbopol gel 934

Optimization of a characterization of tamoxifen loaded transferosomal formulation

The best tofacitinib-loaded transferosomal formulation systems were chosen based on particle size, percent entrapment efficiency, and polydispersivity index, with vesicle size minimized using the point prediction approach in Design Expert Software Version 12. The table below highlights the individual variable composition as well as the overall evaluation. Particles of various sizes were generated based on 27 different transferosomal formulas created with the design expert software version 12. The average size ranged between 118.3 and 546 nm. Phosphatidylcholine (60mL), sodium deoxycholate (40mg), and a solvent mixture of chloroform and methanol (3:1), along with 100 mg of tofacitinib, were determined to meet the requirements of an optimum formulation F2 (60, 40, 2 concentration). Design expert software version 12 was used to generate response 3D graphs. These plots were used to investigate the effects of three different
independent factors on the replies while keeping the fourth variable constant as shown in the table 4.

**Evaluation studies of transfersomal tofacitinib citrate**  
**Formulation in carbopol-934 gel**  
**Transmission electron microscopy**

Transmission electron micrographs are depicted in the image. They showed that the vesicles formed are nano-sized and unilamellar in nature. The contour and core of well-identified spherical vesicles, indicating the conservation of sealed vesicular structure and proving vesicular qualities. The vesicles are smaller unilamellar vesicles with a more uniform size distribution.

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>APPEARANCE</th>
<th>GRITTINESS</th>
<th>SPREADABILITY (GM.CM/SEC.)</th>
<th>EXTRUDABILITY</th>
<th>VISCOSITY</th>
<th>% DRUG CONTENT</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG1</td>
<td>White and opaque</td>
<td>No</td>
<td>3.24</td>
<td>6.2±0.32</td>
<td>610.46±0.32</td>
<td>83.84±0.45</td>
<td>6.6</td>
</tr>
<tr>
<td>TG2</td>
<td>Highly viscous</td>
<td>No</td>
<td>2.56</td>
<td>7.7±0.24</td>
<td>545.32±0.75</td>
<td>87.34±0.29</td>
<td>6.9</td>
</tr>
<tr>
<td>TG3</td>
<td>Clear and soft</td>
<td>No</td>
<td>1.83</td>
<td>6.3±0.18</td>
<td>424.84±0.35</td>
<td>92.21±0.46</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Values are average of triplicate values ± standard deviation

![Figure 7](image.png)

**Figure 7. Transmission Electron Micrograph of Optimized Transferosomal Gel Formulation**

**Scanning electron microscopy**

The scanning electron micrograph seems spherical and smooth.
X-ray Diffraction Analysis

The X-ray diffractogram of tofacitinib demonstrated a significant diffraction peak at 2 value of the produced transferosomal gel. In the figure, the crystalline peak of the tofacitinib citrate combination was clearly evident. The transfersome formulation, on the other hand, showed a deformed peak for tofacitinib, indicating a relative decline in diffraction intensities. The appearance of a significant peak is very suggestive of the use of pure Tofacitinib in the experiments. Each sample is amorphous in terms of crystallinity, indicating that it is likely to be complexed.

In vitro Drug Release study

The graph displays the results of an in-vitro release curve of Tofacitinib-loaded transfersomes, pure drug suspension, and marketed product in a pH 5.5 phosphate buffer at 37 0.5°C. The percent cumulative drug release of optimized transfersomes, pure drug suspension, and marketed product dispersion in 24 hours was 61.20, 70.42, and 74.34, respectively, in our studies. The results show
that the formulation represents a burst release phase, with about 10-15 percent observed within 2 hours due to drug desorption and release from the transfersomes surface. However, the release of medication from optimized transfersomes was delayed beyond two hours, indicating a persistent release pattern. The findings of the in-vitro release revealed that the burst release of medication was caused by the availability of free apigenin on the transfersome’s outer surface. The drug’s prolonged release was caused by apigenin, which could be the explanation for the drug’s sustained release from the internal lipid phase after the first burst release. Table 7 summarizes the drug release results.

Table 6
Cumulative drug release of different formulations

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TIME INTERVAL</th>
<th>CUMULATIVE DRUG RELEASE OF DIFFERENT FORMULATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PURE DRUG SUSPENSION</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>15.3±0.45</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>23.3±0.34</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>29.3±0.65</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>30.2±0.23</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>49.3±0.54</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>59.2±0.33</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>73.3±0.78</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>86.3±0.21</td>
</tr>
</tbody>
</table>

Values are average of triplicate values ± standard deviation

Figure 10. Cumulative drug release of different formulations
Release Kinetics of Tofacitinib Citrate Loaded Transferosomes

The release kinetics of Tofacitinib Loaded Transferosomes from an improved formulation were compared to several kinetic models. The model was best fitted using data in the Higuchian equation, according to the results. Based on Fickian diffusion, this model describes drug release from an insoluble matrix in a time-dependent manner. The slope of the appropriate plots was used to calculate the release constant, and the regression coefficient was determined. Furthermore, the collected values demonstrated that the first-order kinetics model had the best linearity following the Higuchian model.

![Higuchi Equation](image)

Figure 11. Drug release kinetics of tofacitinib citrate loaded transferosomal gel (TG3)

Stability studies

According to the stability studies, there was a negligible increase in particle size from 118.30.522 to 120.553.86 during the storage conditions (4°C and 25°C). The optimized transfersomes' initial percent Entrapment efficiency was found to be 84.24 0.38 %. It was discovered that after 6 months of storage at 4°C and 25°C, it was 81.050.62 5 and 79.240.45 %, respectively. In contrast, there were minimum significant changes in the percent EE during formulation storage at 4°C and 25°C for 6 months. For six months, the optimized formulation was shown to be stable at 4°C and 25°C temperatures. Table 8 displays the information.
Table 8
Stability studies of different formulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial values 0 month</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(4º±2ºC, 75%RH)</td>
<td>25±2ºC, 60±5% RH</td>
<td>(4º±2ºC, 75%RH)</td>
<td>25±2ºC, 60±5% RH</td>
</tr>
<tr>
<td>Mean particle size %</td>
<td>118.4 ±0.3</td>
<td>118.3 ±0.52</td>
<td>118.4 ±0.3</td>
<td>119.3 ±0.3</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>5±0.3</td>
<td>54</td>
<td>119.4±4.1</td>
</tr>
<tr>
<td>Drug content %</td>
<td>97.35 ±0.35</td>
<td>97.20%</td>
<td>96.52%</td>
<td>97.02%</td>
</tr>
</tbody>
</table>

Values are average of triplicate values ± standard deviation

Table 9
Permeation Parameters Of Tofacitinib Loaded Transferosomes Across Goat Skin

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Transdermal flux (μg/cm²/h)</th>
<th>Permeability coefficient (cm/h)</th>
<th>Lag time (hr)</th>
<th>Diffusion coefficient (cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug gel</td>
<td>5.27</td>
<td>0.21</td>
<td>2.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Marketed formulation gel</td>
<td>5.98</td>
<td>0.287</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>Transferosomal gel</td>
<td>7.53</td>
<td>0.258</td>
<td>1.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>
Skin Retention Study

Skin retention experiments of various formulations were carried out in order to determine the content of tofacitinib in the skin after 24 hours of diffusion. The study showed that percentage drug retention of formulations was found higher for TG as compared to pure drug (PDT). The % retention was near about similar for Marketed formulation, 0.68±0.32 and that of plain drug loaded gel was 0.63±0.28 and Transfersomal gel was 0.80±0.05. Skin penetrating effect of the carbopol based novel gel formulations (TG) were studied. The result showed that the nanovesicular moieties embedded in the gel has the ability to penetrate deeper in to the dermal layer and exert theirs depot effect. The polymeric shell also facilitates its entry into the cells. The percent drug retention of the TG formulation after 24 hours was satisfactory in comparison to the marketed formulation.

Table 10
Skin Retention Study

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>RETENTION OF DRUG AFTER 24 HRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug gel</td>
<td>0.68±0.28</td>
</tr>
<tr>
<td>Marketed gel formulation</td>
<td>0.72±0.32</td>
</tr>
<tr>
<td>Transfersomal gel</td>
<td>0.85±0.05</td>
</tr>
</tbody>
</table>

Values represent as mean ± S D (n=3)
Skin cancer is spreading at an alarming rate and becoming resistant to traditional treatments necessitating improved drug delivery system. Vesicular carriers demonstrate to be very promising novel drug delivery units in terms of biocompatibility, reduced toxicity, and enhanced sustained release quality, which would be critical in addressing issues pertaining to compromised therapeutic efficacy of bio-actives, particularly through topical route of administration. Transfersomes cross the skin barrier by opening extracellular channels between organ cells and then deforming to fit into such passages. Transfersomes go through a series of stress-dependent modifications to the local carrier composition to reduce resistance to travel through otherwise restricting pathways. Tofacitinib carbopol-based transfersomal gel was created using the DOE thin film hydration process and assessed for the treatment of skin cancer in an ex vivo permeation study. According to the findings, sodium deoxycholate appears to be superior in terms of vesicle size and entrapment efficiency. When compared to marketed formulations such as standard tamoxifen gel and pure drug dispersion, the transfersomal gel improved both in vitro skin permeability and skin deposition of Tofacitinib. It was shown to be non-irritating to the skin and capable of retaining the drug Tofacitinib in the deeper layers of the skin for a longer period of time. Skin retention time of transfersomal gel was increased two folds as when compared to the other conventional dosage forms. As a result, it is concluded that high molecular weight medications can also conform to the skin in order to reach the targeted place via transfersomes. Transfersomes may achieve new heights in the transdermal medication delivery system in the future.

**Acknowledgement**

We are grateful to the heads of the Department of Pharmaceutics for their unlimited cooperation and support in data collection process. Special thanks to
my guide for the continuous moral support and encouragement. Sincere thanks to NRC and Nanotechnology department.

**Funding**

Nil

**Authors Contributions**

All the authors contributed equally.

**Consent for publication**

Not applicable.

**Conflict of interests**

Declared none

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**References**


