Proceeding Paper

Development and Evaluation of Ebastine-Loaded Transfersomal Nanogel for the Treatment of Urticaria (Autoimmune Disease) †

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Abstract: Urticaria is an autoimmune disease and many patients are suffering from it. This research aims to investigate the development and characterization of an Ebastine-loaded transfersomal nanogel for the enhancement of bioavailability in the treatment of urticaria. The flexible transfersomes, consisting of the drug Ebastine, soya lecithin, and edge activator Tween 80, were prepared using the thin-film hydration method. The transfersomal nanogel was formulated by using the dispersion method and a suitable concentration of the gelling agent Carbopol 934. The transfersomes and their gel were evaluated for various parameters. The Ebastine-loaded transfersomes showed the highest entrapment efficiency, up to 79.92%. The polydispersity index (PDI) of the transfersomes was determined to be 0.103, and the zeta potential was determined to be $-18.9 \text{ mV}$, indicating that the formulation was stable. The drug content of the transfersome gel was found to be 83.67%. The transfersomal gel formed using 1% Carbopol 934 showed the best results, showing in vitro release for up to 8 h and following a zero-order kinetic model. As per the microbial studies conducted, the Ebastine transfersomal gel has a good anti-microbial effect against S. aureus. These vesicular transfersomes are more flexible than other vesicular systems, making them excellent for skin penetration. In the future, this will be the best possible approach for the delivery of drugs via the transdermal route.

Keywords: urticaria; ebastine; Carbopol 934; S. aureus

1. Introduction

Recent research has focused on the development of novel medication delivery techniques with the primary goal of improving patient compliance and therapeutic activity. Although many drug delivery strategies with better therapeutic action have been devised, not all of them are without challenges [1]. Oral medications are exposed to a hostile environment in the GI tract, where most pharmaceuticals metabolize under alkaline or acidic conditions, with solubility difficulties, and most significantly, undergo first-pass metabolism. Parenteral preparation has a variety of drawbacks, including a lack of medication reversal, hypersensitivity, infection risk, and cost [2,3].

Gregor Cevc coined the term “Transfersome” and the underlying notion in 1991. A transfersome is a complex agglomeration that is highly flexible and stress-responsive. Its preferred form is an ultra-deformable vesicle with a highly complex lipid bilayer encasing an aqueous core. Because the bilayer’s local composition and form are interdependent, the vesicle self-regulates and self-optimizes. This allows the transfersome to effortlessly negotiate a variety of transportation hurdles while also serving as a drug carrier for non-invasive targeted medicine administration and the continuous release of therapeutic chemicals [4].

Ebastine is a non-sedating, long-acting, second-generation histamine H1 receptor antagonist used to treat atopic dermatitis, chronic idiopathic urticaria, allergic rhinitis, and...
chronic idiopathic urticaria. It is a BCS class II medicine with poor oral bioavailability. Urticaria is common around the world, with 12–22% of the population encountering it at least once in their lives [5]. The prevalence of urticaria in men and women varies according to research; however, it is more common in women than in men, ranging from 31 to 53%. Urticaria can appear in persons of all ages. Wheals and flares can emerge within hours (or even minutes). Hives arise in episodes that can last a day, weeks, or months, depending on the allergen [6].

The aim of the present study was to formulate and evaluate an Ebastine-loaded transfersomal nanogel and characterize it in vitro. The goal was to improve the bioavailability of Ebastine by incorporating it into a transfersomal nanogel formulation.

2. Materials and Methods

Ebastine was received as a gift sample from Micro Labs Pvt. Ltd., Mumbai, Maharashtra, India. Soya lecithin, Tween 80, Span 60, Carbopol 934, Dichloromethane, Triethanolamine, Methyl Paraben, and Propyl Paraben were purchased from Research-Lab Fine Chem Industries, Mumbai, Maharashtra, India.

2.1. Preparation of Ebastine-Loaded Transfersomes

A thin film is generated by dissolving a mixture of phospholipids and surfactants that form vesicles in an organic solvent (dichloromethane) (Table 1). The organic solvent is subsequently evaporated using a rotary evaporator (Superfit Rotavap—PBU 6D Mumbai, India) at 60 °C. By rotating at 60 rpm for 1 h at the corresponding temperature, a thin film hydrated with a buffer (pH 7.4) was formed, which was kept overnight to allow the vesicles to swell. The resultant vesicles were sonicated at room temperature for 30 min using a bath sonicator or probe sonicator to prepare tiny vesicles (Figure 1) [7].

2.2. Preparation of Ebastine-Loaded Transfersomal Nanogel

The most effective transfersome formulation was chosen for incorporation into the gel system using the dispersion technique with various concentrations of Carbopol 934 (Table 2). A suitable amount of Carbopol 934 was sprinkled in the distilled water while continuously stirring on a magnetic stirrer (REMI 1MLH, International Scientific Instruments Co., Delhi, India), which was then soaked and hydrated. Other chemicals, such as Propylene Glycol, as well as the required amount of drug entrapped in transfersome, were then added and uniformly disseminated with continuous stirring. Triethanolamine was used to neutralize the nanogel to pH 7 (pH is acceptable for skin), and the final weight was adjusted using distilled water [9].

The transfersomal nanogel was evaluated for pH, viscosity, spreadability, extrudability, drug content, and various other characteristics, and an anti-microbial study was conducted against *S. aureus* by using the agar well diffusion method [10,11].

Table 2. Formulation table of Ebastine-loaded transfersomal nanogel

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Carbopol 934 (%)</th>
<th>Propylene Glycol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF2G1</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>TF2G2</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>TF2G3</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>TF2G4</td>
<td>2.0</td>
<td>5</td>
</tr>
</tbody>
</table>

3. Results and Discussion

UV spectrophotometry (Shimadzu—UV 1800) was used for the determination of λ\textsubscript{max} and plotting of the calibration curve of the drug in methanol and in phosphate buffer (pH 7.4) for the confirmation of the drug.

In the methanol and phosphate buffer, pH 7.4, λ\textsubscript{max} of Ebastine was detected at 253 nm and 257 nm, respectively. The compatibility between the drug and the excipients was confirmed using the FTIR method. The spectrum of Ebastine was recorded using FTIR (Shimadzu IR Affinity-1S CE). The FTIR spectrum of pure Ebastine showed major peaks at 2943 cm\(^{-1}\) and 2818 cm\(^{-1}\) with C-H stretching, at 1677 cm\(^{-1}\) [8].

*Figure 1.* Schematic representation of thin-film hydration method.


Table 1. Formulation table for Ebastine-loaded transfersomes.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug (mg)</th>
<th>Soya lecithin (%)</th>
<th>Tween 80 (%)</th>
<th>Span 60 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>100</td>
<td>95</td>
<td>05</td>
<td>-</td>
</tr>
<tr>
<td>TF2</td>
<td>100</td>
<td>90</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>TF3</td>
<td>100</td>
<td>85</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>TF4</td>
<td>100</td>
<td>80</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>TF5</td>
<td>100</td>
<td>95</td>
<td>-</td>
<td>05</td>
</tr>
<tr>
<td>TF6</td>
<td>100</td>
<td>90</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>TF7</td>
<td>100</td>
<td>85</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>TF8</td>
<td>100</td>
<td>80</td>
<td>-</td>
<td>20</td>
</tr>
</tbody>
</table>

The prepared transfersomes were optimized using metrics such as entrapment efficiency, TEM analysis, polydispersibility index, and zeta potential. Optimized transfersomes were used for further characterization [8].

2.2. Preparation of Ebastine-Loaded Transfersomal Nanogel

The most effective transfersome formulation was chosen for incorporation into the gel system using the dispersion technique with various concentrations of Carbopol 934 (Table 2). A suitable amount of Carbopol 934 was sprinkled in the distilled water while continuously stirring on a magnetic stirrer (REMI 1MLH, International Scientific Instrument Co., Delhi, India), which was then soaked and hydrated. Other chemicals, such as Propylene Glycol, as well as the required amount of drug entrapped in transfersome, were then added and uniformly disseminated with continuous stirring. Triethanolamine was used to neutralize the nanogel to pH 7 (pH is acceptable for skin), and the final weight was adjusted using distilled water [9].

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<td>TF2G2</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>TF2G3</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>TF2G4</td>
<td>2.0</td>
<td>5</td>
</tr>
</tbody>
</table>

The transfersomal nanogel was evaluated for pH, viscosity, spreadability, extrudability, drug content, and various other characteristics, and an anti-microbial study was conducted against S. aureus by using the agar well diffusion method [10,11].

3. Results and Discussion

UV spectrophotometry (Shimadzu—UV 1800) was used for the determination of \( \lambda_{\text{max}} \) and plotting of the calibration curve of the drug in methanol and in phosphate buffer (pH 7.4) for the confirmation of the drug. In the methanol and phosphate buffer, pH 7.4, \( \lambda_{\text{max}} \) of Ebastine was detected at 253 nm and 257 nm, respectively. The compatibility between the drug and the excipients was confirmed using the FTIR method. The spectrum of Ebastine was recorded using FTIR (Shimadzu IR Affinity-1S CE). The FTIR spectrum of pure Ebastine showed major peaks at 2943 cm\(^{-1}\) and 2818 cm\(^{-1}\) with C-H stretching, at 1677 cm\(^{-1}\) with C=O stretching, and at 1357 cm\(^{-1}\) with O-H bending. Soya Lecithin C-H stretching appeared at 2854 cm\(^{-1}\) and 1735 cm\(^{-1}\) showing C=O stretching. Tween 80 C-H stretching appeared at 2870 cm\(^{-1}\), and C=O stretching at 1736 cm\(^{-1}\). Carbopol 934 showed C-H stretching at 2916.37 cm\(^{-1}\) and 2854.65 cm\(^{-1}\), C=O stretching at 1697.36 cm\(^{-1}\), and C-H bending at 1458.18 cm\(^{-1}\). The preformulation study shows that there are no potential interactions between drugs and excipients.
3.1. Characterization of Ebastine-loaded Transfersomes

3.1.1. Entrapment Efficiency

Transfersomes containing Ebastine were separated from unentrapped drugs via centrifugation (Remi C-24 plus) at 10,000 rpm for 30 min at 4 °C. The supernatant was recovered and assayed spectrophotometrically at 253 nm using a Shimadzu UV-Vis double-beam spectrophotometer (Shimadzu-1800, Shimadzu, Kyoto, Japan). The highest entrapment efficiency of 79.92 ± 1.19% was shown by formulation TF2 compared to the other formulations.

3.1.2. Transmission Electron Microscopy (TEM)

Transmission electron microscopy (Tecnai G2 spirit Biotwin) was used for the visualization of transfersomes vesicles. The TEM image (Figure 2) of vesicles shows a particle size of 200–300 nm, which is ideal for transfersome delivery via the skin.

3.1.3. Zeta Potential

The zeta potential of the TF2 formulation was calculated using a zeta-sizer (Malvern zeta sizer, Malvern Instrument Ltd., Worcestershire, United Kingdom). The zeta potential was determined to be −18.9 ± 4.84 mV, which indicates that the transfersomes were stable (Figure 3a).

3.1.4. Size Distribution and Polydispersity Index (PDI)

Size distribution and polydispersity index (PDI) were determined using a zeta-sizer (Malvern zeta sizer). The average size of transfersomes was found to be 240 ± 91.21 nm, and the polydispersity index (PDI) was found to be 0.103 (Figure 3b).

3.2. Characterization of Ebastine-Loaded Transfersomal Nanogel

3.2.1. Homogeneity and Grittiness

Formulation TF2G2 has shown better homogeneity compared to other transfersomal formulations.

3.2.2. Determination of pH

The pH of the transfersomal nanogel was found to be in the range of 7.4–7.35. This range of pH is acceptable for the skin (Table 3).

3.2.3. Viscosity

The viscosity of the transfersomal nanogel was evaluated using a Brookfield viscometer (Shimadzu-1800, Shimadzu, Kyoto, Japan). The highest viscosity was measured using a small amount of shear. The spreadability of TF2G2 was found to be better compared to other formulations (Table 3).

3.2.4. Spreadability

The term spreadability indicates the ease with which the nanogel spreads easily by the application of a small amount of shear. The spreadability of TF2G2 was found to be better compared to other formulations (Table 3).

3.2.5. FTIR Spectroscopy

The FTIR spectrum of the transfersomal nanogel showed typical peaks at 2916.37 cm⁻¹ and 2854 cm⁻¹ showing C=O stretching. Tween 80 C-H stretching appeared at 2870 cm⁻¹, and C=O stretching at 1736 cm⁻¹. Carbopol 934 showed C-H stretching at 2924 cm⁻¹ and 2852 cm⁻¹ showing O-H bending. Soya Lecithin C-H stretching appeared at 2854 cm⁻¹ with O-H bending. Soya Lecithin C=O stretching appeared at 1736 cm⁻¹, and C=O stretching at 1697.36 cm⁻¹. The preformulation study shows that there are no potential interactions between drugs and excipients.

3.2.6. Drug Content

The drug content of the transfersomal nanogel was determined to be 1458.18 cm⁻¹ and 1357 cm⁻¹ with C=O stretching, and at 1357 cm⁻¹ with O-H bending. Soya Lecithin C-H stretching appeared at 2854 cm⁻¹, and C=O stretching at 1736 cm⁻¹. Carbopol 934 showed C-H stretching at 2924 cm⁻¹ and 2852 cm⁻¹ showing O-H bending. Soya Lecithin C=O stretching appeared at 1736 cm⁻¹, and C=O stretching at 1697.36 cm⁻¹. The preformulation study shows that there are no potential interactions between drugs and excipients.

Table 3: Drug content of transfersomal nanogel formulations.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gel</th>
<th>Mean (mg/mL)</th>
<th>Area (%)</th>
<th>St Dev (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation TF2G2</td>
<td>76.24 ± 3.11</td>
<td>100.0</td>
<td>5.32</td>
<td></td>
</tr>
<tr>
<td>Formulation TF2G1</td>
<td>65.37 ± 2.78</td>
<td>100.0</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>Formulation TF2G4</td>
<td>46 ± 1.5</td>
<td>100.0</td>
<td>4.19</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. (a) Zeta potential; (b) polydispersity index of Ebastine-loaded transfersomal formulation.
3.1.4. Size Distribution and Polydispersity Index (PDI)

Size distribution and polydispersity index (PDI) were determined using a zeta-sizer (Malvern zeta sizer). The average size of transfersomes was found to be 240 ± 91.21 nm, and the polydispersity index (PDI) was found to be 0.103 (Figure 3b).

3.2. Characterization of Ebastine-Loaded Transfersomal Nanogel

3.2.1. Homogeneity and Grittiness

Formulation TF2G2 has shown better homogeneity compared to other transfersomal nanogel. Formulation TF2G2 showed no presence of any particulate matter.

3.2.2. Determination of pH

A gel weighing 1 g was dissolved in 25 mL of distilled water at 25 °C, and the pH level was measured using a digital pH meter (Equiptronics—EQ 610, Mumbai, India). The pH of the transfersomal nanogel was found to be in the range of 7.4–7.35. This range of pH is acceptable for the skin (Table 3).

Table 3. Results of evaluation of Ebastine-loaded transfersomal nanogel.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Viscosity (cP)</th>
<th>Spreadability (g.cm/s)</th>
<th>Extrudability (%)</th>
<th>Drug Content (%)</th>
<th>Drug Deposition (%)</th>
<th>Gel Strength (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF2G1</td>
<td>7.4</td>
<td>40,191 ± 246</td>
<td>74.83 ± 5.71</td>
<td>90.04 ± 2.12</td>
<td>76.24 ± 3.11</td>
<td>19.32 ± 2.39</td>
<td>34 ± 1.2</td>
</tr>
<tr>
<td>TF2G2</td>
<td>7.16</td>
<td>43,970 ± 324</td>
<td>76.56 ± 5.32</td>
<td>96.02 ± 3.61</td>
<td>83.67 ± 3.81</td>
<td>15.45 ± 2.93</td>
<td>38 ± 1.4</td>
</tr>
<tr>
<td>TF2G3</td>
<td>7.35</td>
<td>47,326 ± 427</td>
<td>55.42 ± 4.48</td>
<td>93.00 ± 3.72</td>
<td>72.53 ± 4.13</td>
<td>23.12 ± 3.15</td>
<td>43 ± 1.1</td>
</tr>
<tr>
<td>TF2G4</td>
<td>7.14</td>
<td>54,123 ± 521</td>
<td>41.20 ± 4.19</td>
<td>88.31 ± 4.11</td>
<td>69.82 ± 4.51</td>
<td>26.78 ± 3.38</td>
<td>46 ± 1.5</td>
</tr>
</tbody>
</table>

3.2.3. Viscosity

The viscosity of the transfersomal nanogel was evaluated using a Brookfield viscometer (DV2T model, Brookfield Engineering Laboratories, Middleboro, MA, USA) with a Helipath T spindle (D94). TF2G2 had a suitable viscosity when compared to other formulations (Table 3).

3.2.4. Spreadability

The term spreadability indicates the ease with which the nanogel spreads easily by the application of a small amount of shear. The spreadability of TF2G2 was found to be better compared to other formulations (Table 3).

3.2.5. Extrudability

All the formulations had good extrudability in the range of 96.02 ± 3.61% to 88.31 ± 4.11%. Formulation TF2G2 showed the highest extrudability compared to other formulations (Table 3).

3.2.6. Drug Content

Formulation TF2G2 showed the highest drug content: 83.67 ± 3.81%. The drug content was found to be in the range of 69.82 ± 4.51% to 83.67 ± 3.81% (Table 3).

3.2.7. Gel Strength

The gel strength indicates the gel’s tensile strength. This demonstrates the ability of the gelled mass to withstand external pressure. All of the formulations had good gel strength, with values ranging from 34 ± 1.2 s for TF2G1 to 46 ± 1.5 s for TF2G4 (Table 3).

3.2.8. Drug Deposition

The amount of medication deposited on the transdermal layer after 24 h of diffusion was found to be lowest for TF2G2, i.e., 15.45 ± 2.93%, indicating that 83.67 ± 3.81% of the
drug was released during diffusion. As a result, we can conclude that TF2G2 is preferable to the other formulations (Table 3).

3.2.9. In Vitro Release and Kinetic Modelling

The TF2G2 formulation showed the maximum drug release of up to $84.54 \pm 6.82\%$ at 8 h. The kinetic studies indicate that the TF2G2 formulation follows a zero-order model.

3.2.10. Anti-Microbial Study

The antimicrobial activity was evaluated by measuring the zone of inhibition on *S. aureus*. The zone of inhibition on *S. aureus* of pure Ebastine and Ebastine-loaded transfersomal nanogel was found to be $36 \pm 0.31$ mm and $41 \pm 0.22$ mm, respectively.

3.2.11. Stability Studies

The optimized transfersomal nanogel formulation (TF2G2) was stored at $40 \pm 2 ^\circ C/75\%$ RH in a stability chamber for 90 days. The sample was withdrawn periodically and evaluated for pH, % drug content, and in vitro drug diffusion, which was found to be optimum and satisfactory, and there was no significant change in the formulation.

4. Conclusions

The Ebastine-loaded transfersome demonstrated the highest entrapment efficiency of up to $79.92 \pm 1.19\%$. The transfersomes’ polydispersity index (PDI) was 0.103 and their zeta potential was $-18.9 \pm 4.84$ mV, indicating that the formulation was stable. The transfersomal nanogel’s drug content was found to be $83.67 \pm 3.81\%$. The best results were obtained using a transfersomal nanogel made with 1% Carbopol 934, which demonstrated in vitro release for up to 8 h and followed a zero-order kinetic model. As per the microbial studies conducted, the Ebastine transfersomal nanogel showed good anti-microbial effects against *S. aureus*, indicating its usage for urticaria. These vesicular transfersomes are more flexible than other vesicular systems, thereby making them ideal for skin penetration.


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Conflicts of Interest: The authors declare no conflict of interest.

References


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