

Proceeding Paper

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

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Abstract: Urticaria it is an autoimmune disease and many patients are suffering from it. The research aims to investigate the development and characterization of Ebastine-loaded transfersomal nanogel for 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 with the thin film hydration method. The transfersomal nanogel was formulated by using the dispersion method and utilizing a suitable concentration of the gelling agent Carbopol 934. Transfersomes and its gel were evaluated for various parameters. The Ebastine loaded transfersome 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 to be -18.9 mV, indicating that the formulation was stable. The drug content of the transfersome gel was found to be 83.67%. Transfersomal gel formed using 1% Carbopol 934 showed the best results, showing in-vitro release 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 impact against *S. aureus*. These vesicular transfersomes are more flexible than other vesicular systems, making them excellent for skin penetration. In future it will be a best possible approach for the delivery of drug via transdermal route.

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

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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. While 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 metabolise under alkaline or acidic conditions, have 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 is self-regulating and self-optimizing. 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 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 a 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 31–53%. 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 present study was to formulate and evaluate ebastine loaded transfersomal nanogel and its in-vitro characterization. The is to improve its bioavailability of ebastine by incorporating it in 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, 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) at 60 °C. By rotating at 60 rpm for 1 h at the corresponding temperature, a thin film hydrated with buffer (pH 7.4) was formed. Keep it 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].

Table 1. Formulation table for ebastine loaded transfersomes.

Formulation	Drug (mg)	Soya lecithin (%)	Tween 80 (%)	Span 60 (%)
TF1	100	95	05	-
TF2	100	90	10	-
TF3	100	85	15	-
TF4	100	80	20	-
TF5	100	95	-	05
TF6	100	90	-	10
TF7	100	85	-	15
TF8	100	80	-	20

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

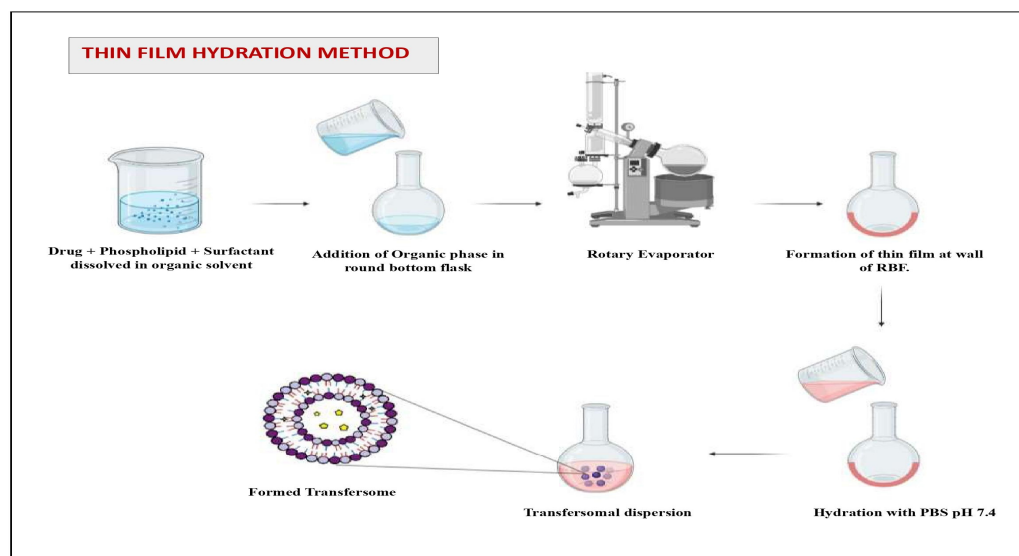


Figure 1. Schematic representation of Thin Film Hydration Method.

2.2. Preparation of Ebastine Loaded Transfersomal Nanogel

The most effective transfersome formulation was chosen for incorporation in the gel system made by the Dispersion technique with varied concentrations of Carbopol 934. A suitable amount of Carbopol 934 was sprinkled in the distilled water while continuously stirring on a magnetic stirrer (REMI 1MLH), and it was then soaked and hydrated. Other chemicals such as Propylene Glycol, as well as the needed 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 characteristics and anti-microbial study was conducted against *S. aureus* by using agar well diffusion method [10,11].

Table 2. Formulation table of ebastine loaded transfersomal nanogel.

Formulation	Carbopol 934 (%)	Propylene Glycol (%)
TF2G1	0.5	5
TF2G2	1.0	5
TF2G3	1.5	5
TF2G4	2.0	5

3. Results and Discussion

UV spectrophotometry (Shimadzu—UV 1800) was used for determination of λ_{\max} and plotting of calibration curve of drug in methanol and in phosphate buffer pH 7.4 for the confirmation of drug. In methanol and phosphate buffer pH 7.4, λ_{\max} of Ebastine was detected at 253 nm and 257 nm, respectively. The compatibility between the drug and the excipients was confirmed using FTIR method. The spectrum of the drug Ebastine is recorded using FTIR (Shimadzu IR Affinity-1S CE). The FTIR spectrum of pure Ebastine major peaks 2943 cm^{-1} , 2818 cm^{-1} shows C-H stretching, 1677 cm^{-1} shows C=O stretching, 1357 cm^{-1} shows 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 1697.36 cm^{-1} and C-H bending at 1458.18 cm^{-1} . The preformulation study shows that there are no potential interactions between drug and excipients.

3.1. Characterization of Ebastine loaded Transfersomes

3.1.1. Entrapment Efficiency

Transfersomes containing Ebastine was separated from untrapped drug by centrifugation (Remi C-24 plus) at 10,000 rpm for 30 min at 4 °C. The supernatant was recovered and assayed spectrophotometrically using Shimadzu UV-Vis double-beam spectrophotometer at 253 nm. The highest entrapment efficiency of $79.92 \pm 1.19\%$ was shown by formulation TF2 as compare to other formulations.

3.1.2. Transmission Electron Microscopy (TEM)

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

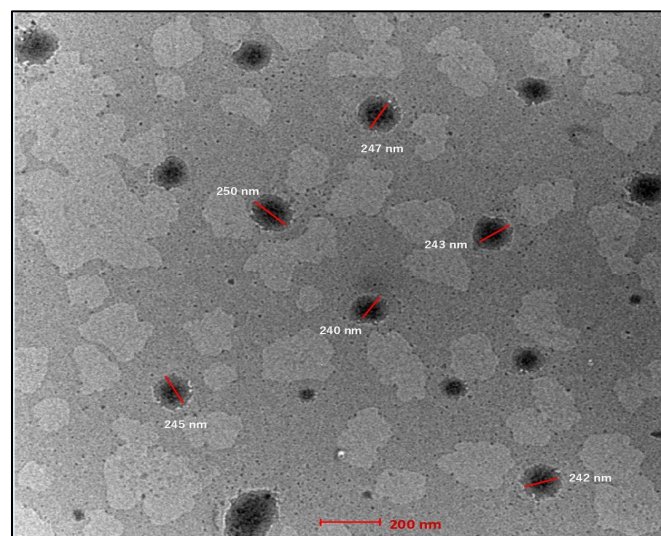


Figure 2. TEM image of transfersome vesicles.

3.1.3. Zeta Potential

The zeta potential of the TF2 formulation was calculated using zeta-sizer (Malvern zeta sizer). The zeta potential was determined to be -18.9 ± 4.84 mV, which indicate the transfersomes were stable (Figure 3a).

3.1.4. Size Distribution and Polydispersity Index (PDI)

Size distribution and poly dispersity index (PDI) was determined by zeta-sizer (Malvern zeta sizer). The average size of transfersome was found to be 240 ± 91.21 nm and polydispersity index (PDI) was found to be 0.103 (Figure 3b).

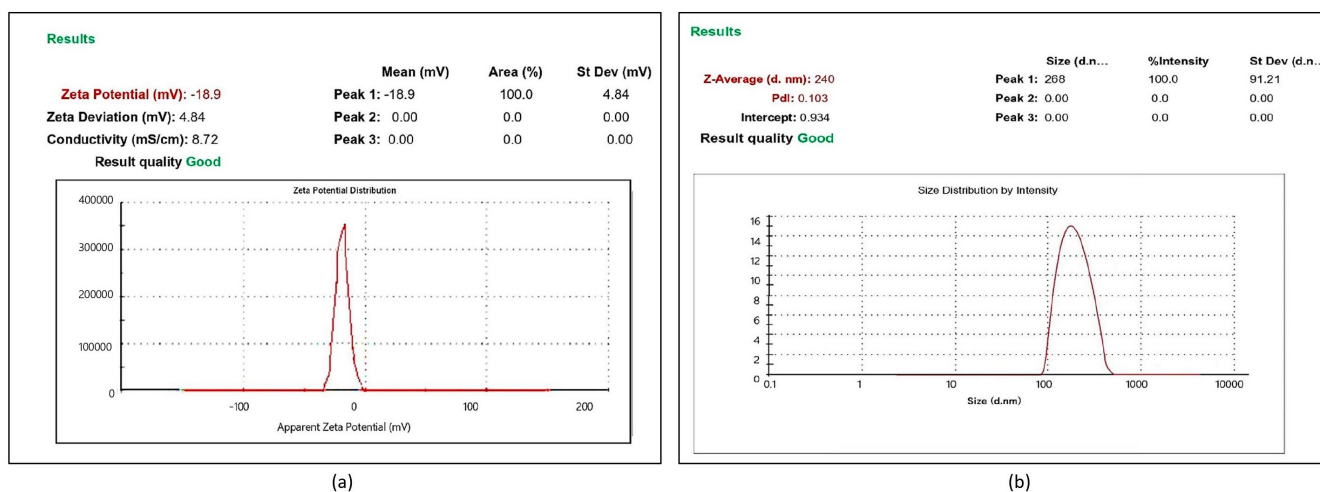


Figure 3. (a) Zeta potential (b) Polydispersity index of ebastine loaded transfersomal formulation.

3.2. Characterization of Ebastine Loaded Transfersomal Nanogel

3.2.1. Homogeneity and Grittiness

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

3.2.2. Determination of pH

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

3.2.3. Viscosity

The viscosity of the transfersomal nanogel was evaluated using a Brookfield viscometer (DV2T model) 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 gets easily spreads by application of small amount of shear. Spreadability of TF2G2 was found to be good as compared to other formulations (Table 3).

3.2.5. Extrudability

All the formulations have good extrudability in range of $88.31 \pm 4.11\%$ to $96.02 \pm 3.61\%$ formulation TF2G2 shows highest extrudability as compared to other formulations (Table 3).

3.2.6. Drug Content

Formulation TF2G2 has shown the highest drug content of $83.67 \pm 3.81\%$. The drug content was found to be in range of $69.82 \pm 4.51\%$ to $83.67 \pm 3.81\%$ (Table 3).

3.2.7. Gel Strength

The gel strength indicates the gel's tensile strength. It 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 sec for TF2G1 to 46 ± 1.5 sec 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., $15.45 \pm 2.93\%$, indicating that $83.67 \pm 3.81\%$ of the drug was released during the diffusion. As a result, we can conclude that TF2G2 is preferable than the other formulations (Table 3).

Table 3. Results of evaluation of ebastine loaded transfersomal nanogel.

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

3.2.9. In-Vitro Release and Kinetic Modelling

The TF2G2 formulation showed the maximum drug release upto $84.54 \pm 6.82\%$ at 8 hr. The kinetic studies states that the TF2G2 formulation follows zero order model.

3.2.10. Anti-Microbial Study

Antimicrobial activity was evaluated by measuring the zone of inhibition against *S. aureus*. The zone of inhibition on *S. aureus* of pure ebastine and ebastine loaded transfersomal nanogel was found to be 36 ± 0.31 mm and 41 ± 0.22 mm respectively.

3.2.11. Stability Studies

The optimized transfersomal nanogel formulation (TF2G2) was stored at 40 ± 2 °C/75% RH in a stability chamber for 90 days. Sample was withdrawn periodically and evaluated for pH, % drug content and in-vitro drug diffusion 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 up to $79.92 \pm 1.19\%$. The transfersomes' polydispersity index (PDI) was 0.103 and their zeta potential was -18.9 ± 4.84 mV, showing that the formulation was stable. The transfersome nanogel's drug content was found to be $83.67 \pm 3.81\%$. The best results were obtained with 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 has shown good anti-microbial impact against *S. aureus*, indicating its usage for urticaria. These vesicular transfersomes are more flexible than other vesicular systems, making them ideal for skin penetration.

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