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# Formulation and evaluation of Ebastine transferosomes

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## Abstract

The present study was focused on formulating and evaluating Ebastine containing Transferosomes formulation for *in vitro* studies. Transferosomes formulations were prepared by using cold method and were evaluated for *in vitro* characteristics, stability studies. Transferosomes formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that Transferosomes formulation was spherical in shape. Transferosomes containing lipid higher percentage of drug release after 8 h as compared to other formulations. F-2 formulation was found to be stable at the end of the study on storage condition. The present study suggested that Transferosomes gel formulations provide sustained and prolonged delivery of drug with enhance bioavailability.

**Keywords:** Transferosomes; Ebastine; Bioavailability; Reverse Phase Evaporation; *In Vitro* Drug Release Studies

## 1. Introduction

Transferosomes are such novel vesicular drug delivery systems whose uniqueness is an ultra-deformable vesicle. It can squeeze itself through a pore, many times smaller than its size owing to its elasticity, designed to enhance the skin penetration and deliver the drug non-invasively through the skin barrier without measurable loss [1]. Transferosomes have been widely used as carrier for the controlled and targeted delivery of proteins, peptides, hormones and several drugs [2]. The oral delivery of peptides such as insulin and interferons, is impossible due to their instability and rapid degradation in the harsh environment of gastro intestinal tract. Ebastine (EBT), is a histamine H1 receptor blocker which is used to treat different allergic diseases. EBT (BCS-II drug) is concerned, its oral bioavailability is about 40% [3]. To obtain maximum therapeutic benefits from EBT, its oral bioavailability should be enhanced. For that purpose, several approaches have been employed by scientists to increase the bioavailability of EBT, such as complexation, nanoparticle formulation, fast disintegrating tablets and micro emulsion, as well as spray drying technique. The objective of this work is to develop TOFs for the delivery of EBT through oral route to achieve improved therapeutic plasma levels [4].

## 2. Material

Ebastine was obtained from Alkem Pvt Mumbai, Soya lecithin, Cholesterol and Span 80 procured from SD fine chemicals Mumbai. Other chemicals and the reagents used were of analytical grade.

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## 3. Methodology

### 3.1. Fourier Transforms Infrared Spectroscopy

Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 450-4000 $cm^{-1}$ and the resolution was 4 $cm^{-1}$.[5]

### 3.2. Formulation Development

**Table 1** Formulation Table

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| Drug | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Soya lecithin | 100 | 62.5 | 25 | 25 | 62.5 | 100 | 62.5 | 25 |
| Cholesterol | 62.5 | 62.5 | 62.5 | 100 | 62.5 | 25 | 25 | 25 |
| Span 80 | 2 | 2 | 2 | 5 | 2 | 5 | 2 | 5 |
| Chloroform | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |

### 3.3. Preparation Method Transferosomes

Transferosomes containing Ebastine were prepared by reverse phase evaporation method with some modification as described in the literature. At first, specified amount of lipids (soya lecithin and cholesterol) and Ebastine were taken in a clean beaker. Then, span 80 as surfactant was poured in the same beaker and dissolved in a solvent mixture of ether and chloroform in the ratio of 3:1. The ratio of lipids and surfactants was maintained 1:1 in all the formulations. The beaker was kept at the room temperature for 24 h until the thin film was formed. Then, the resulting thin film was resuspended with 2 ml of phosphate buffer saline (pH, 7.4) and sonicated by a probe Sonicator (FS-600, Frontline Electronics and Machinery Pvt. Ltd., India) for 5 min at room temperature. The film was hydrated using 2 ml of phosphate buffer saline (pH, 7.4) and then further sonicated for 10 min to obtain highly deformable vesicles as Transferosomes suspensions containing Ebastine [6].

### 3.4. Evaluation

#### *3.4.1. Entrapment Efficiency*

*Entrapment efficiency* (%EE) of Transferosomal vesicles was determined by the centrifugation method. The vesicles were separated in a centrifuge at 15000 rpm for 60 min. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by analysing the vesicles using 7.4 phosphate buffer. The vesicles were broken to release the drug, which were then estimated for the drug content [7]. The absorbance of the drug was noted at 291 nm. From this, the entrapment efficiency was determined by the following equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{entrapped drug}}{\text{total drug added}} \times 100$$

#### *3.4.2. Vesicle Physical Analysis*

The shape, surface characteristics, and size of the Transferosomes were observed by scanning electron microscopy [8].

### 3.5. SEM Analysis

The morphological characteristics of formulated Transferosomes were carried by using Scanning electron microscopy (SEM). A small drop of Transferosomes was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85 $^0$C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120 $^0$c and voltage of 5 kV. [9]

### 3.6. *In Vitro* Drug Release Study

*Ex vitro* release studies were carried out using unjacketed vertical Franz diffusion cells with a diffusional surface area of 6.154 $cm^2$ and 20 mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked in phosphate buffer pH 7.4 Formulation equivalent to 5 mg of Ebastine Transferosomes was placed in the donor compartment. The receptor compartment consisting of PB pH 7.4 was maintained at 37± 2 °C under constant stirring upto 32 hrs. The donor chamber and the sampling port were covered with lid to prevent evaporation during the study. Aliquots of 5 mL were withdrawn periodically at different time intervals (5, 10, 15, 30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 up to 32 hrs) and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically [10].

### 3.7. Kinetics of Drug Release: [11]

To study kinetices data obtained from invitro relesase were plotted in various kinetic models.

*3.7.1. Zero-Order Equation*

$$\% R = Kt$$

This model represents an ideal release profile in order to achieve the pharmacological prolonged action.

First Order Equation

Log % unreleased = Kt / 2.303

This model is applicable to study hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms.

Higuchi Equation

$$\% R = Kt^{0.5}$$

*3.7.2. Korsmeyer-Peppas Equation :*

$$\%R=Kt^{n}$$

This model is widely used, when the release phenomenon could be involved.

### 3.8. Stability Studies [12]

The success of an effective formulation can be evaluated only through stability studies. The prepared Ebastine Transferosomes placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2 $^{o}$c and refrigerator 2-8 $^{o}$c for a period of 3 months.

## 4. Results and discussion

### 4.1. FT-IR Spectrum of Ebastine

FT-IR Spectra of Ebastine and F2 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between drug and excipients.

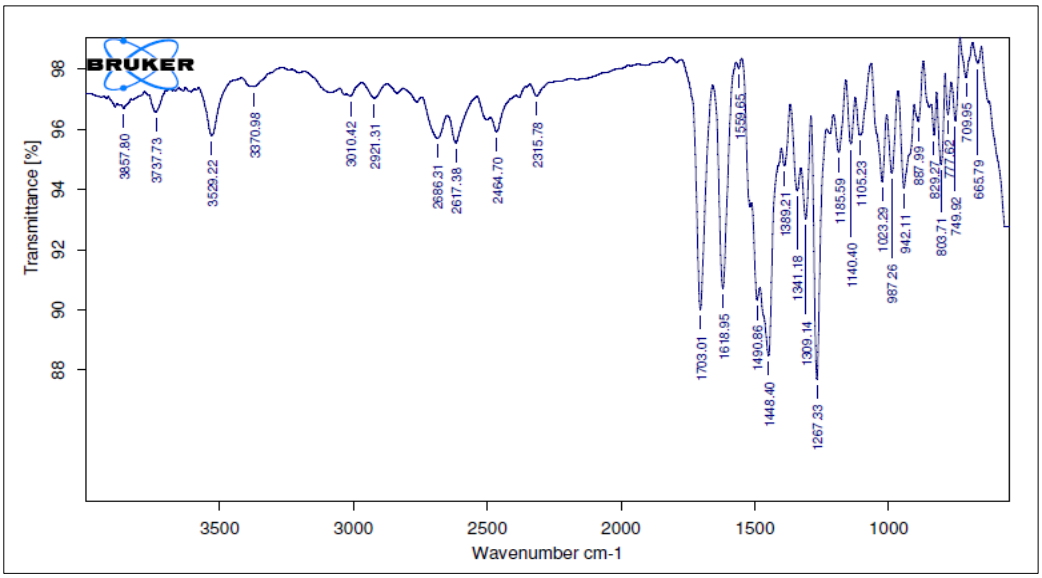


**Figure 1** FT-IR Sample for Ebastine

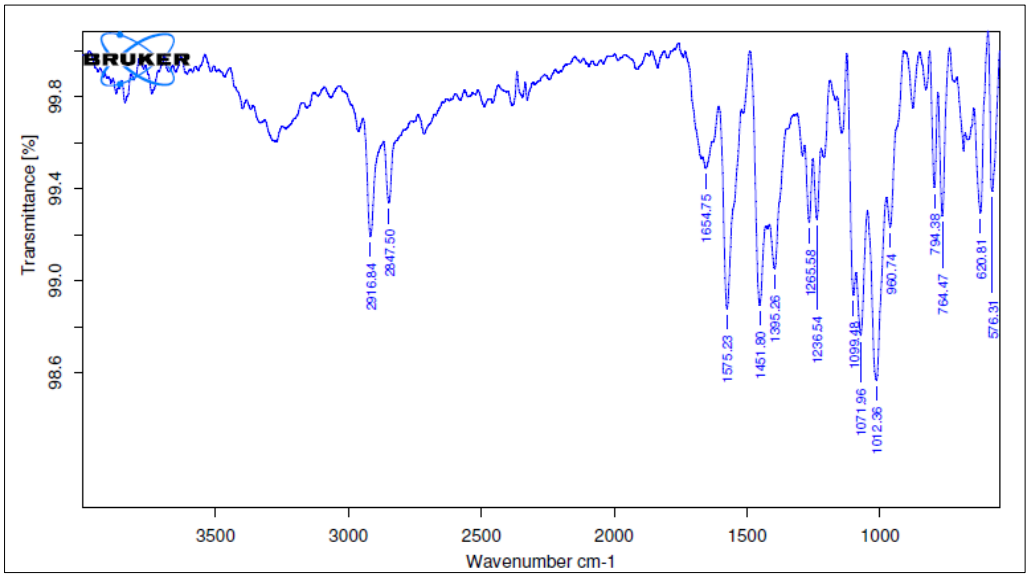


**Figure 2** FT-IR Sample for Physical Mixture of Drug and Excipients

### 4.2. SEM Analysis

Scanning electron microscopy (SEM) SEM revealed that the Ebastine Transferosomes were smooth and spherical without any aggregation.

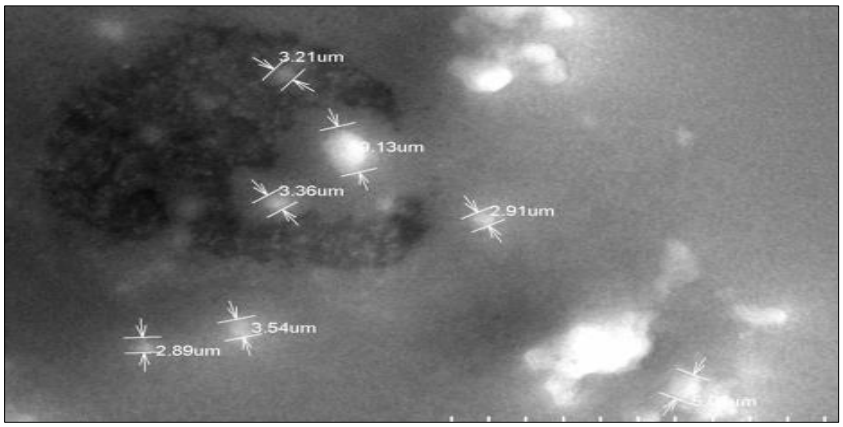


**Figure 3** SEM Analysis of Transferosomes

### 4.3. Entrapment Efficiency

Entrapment efficiency (%EE) of Transferosomes vesicles was determined by the centrifugation method. The vesicles were separated in a centrifuge at 15000 rpm for 60 min. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by analyzing the vesicles using ethanol. The vesicles were broken to release the drug, which were then estimated for the drug content. The absorbance of the drug was noted at 272 nm. From this, the entrapment efficiency was determined by the following equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{entrapped drug}}{\text{total drug added}} \times 100$$

**Table 2** Percentage Entrapment Efficiency

| Ratio | % EE |
|---|---|
| F1 | 89.78 |
| F2 | 90.25 |
| F3 | 88.59 |
| F4 | 79.86 |
| F5 | 78.86 |
| F6 | 88.98 |
| F7 | 79.98 |
| F8 | 75.82 |

### 4.4. *In Vitro Drug* Release Studies

The diffusion medium used was phosphate buffer solution pH 7.4. In the Franz diffusion cell, the egg membrane was mounted between donor and receptor compartment of diffusion cell. Egg membrane separated both the compartments. Area of membrane separating two compartments was 1.7662 $cm^2$. Transferosomal suspension (200 ml) volume was accurately pipetted into donor compartment which was then covered with aluminium foil to avoid any evaporation. Diffusion medium (7 ml) was maintained at 37 ±1 ◦C, so that the membrane just touches the receptor medium surface. A magnetic bar continuously stirred in the diffusion medium at 100 rpm. Each of 0.5 volume was withdrawn at required time intervals and replaced by 0.5 ml volume of receptor medium (phosphate buffer pH 7.4) to maintain the sink condition. These samples were analyzed by UV spectrophotometer at maximum wavelength 291 nm.

**Table 3** Diffusion Studies of Transferosomal Suspension

| Time | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| 0. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 15.6 | 17.0 | 11.8 | 13.6 | 14.6 | 16.1 | 17.9 | 13.8 |
| 2 | 26.9 | 31.1 | 21.6 | 20.8 | 21.8 | 23.7 | 26.8 | 23.4 |
| 3 | 35.8 | 40.7 | 30.9 | 29.1 | 33.6 | 30.9 | 36.1 | 31.5 |
| 4 | 59.8 | 62.9 | 52.7 | 53.8 | 52.8 | 55.8 | 58.1 | 55.9 |
| 6 | 63.4 | 70.6 | 60.8 | 59.6 | 58.6 | 61.5 | 63.8 | 62.4 |
| 8 | 75.1 | 81.7 | 71.4 | 70.8 | 73.1 | 78.2 | 77.6 | 73.8 |
| 10 | 82.3 | 89.4 | 79.8 | 83.1 | 80.1 | 82.5 | 87.5 | 80.3 |
| 12 | 90.5 | 96.3 | 91.2 | 92.6 | 89.9 | 91.8 | 95.6 | 89.1 |

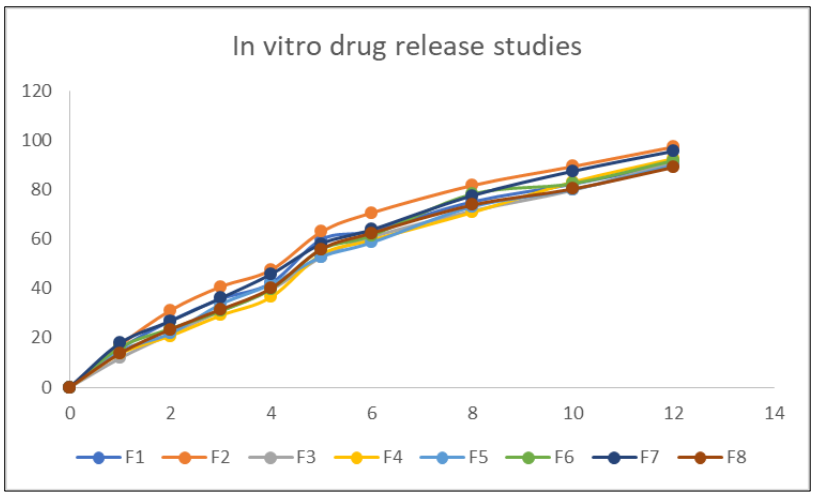


**Figure 4** Diffusion Studies of Transferosomal Suspension

*4.4.1. Zero Order Kinetics*

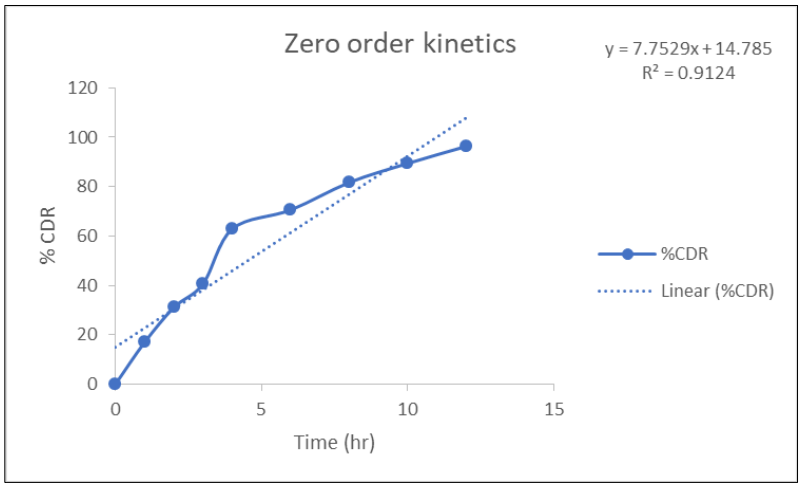


**Figure 5** Zero Order Kinetics Optimized Formulation

*4.4.2. First Order Kinetics*

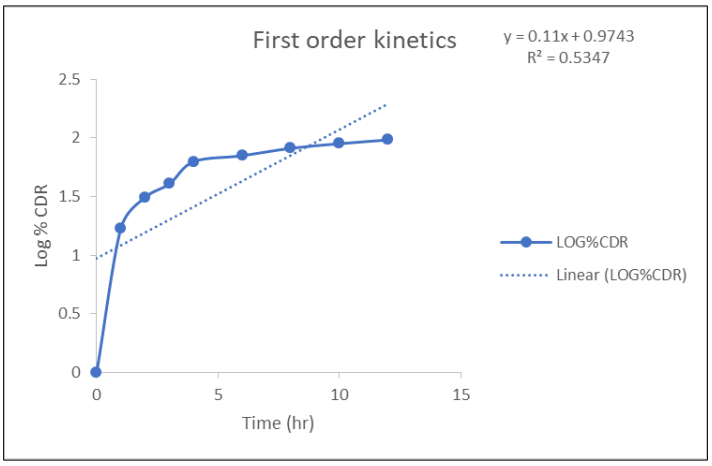


**Figure 6** First Order Kinetics Optimized Formulation

*4.4.3. Higuchi Model*

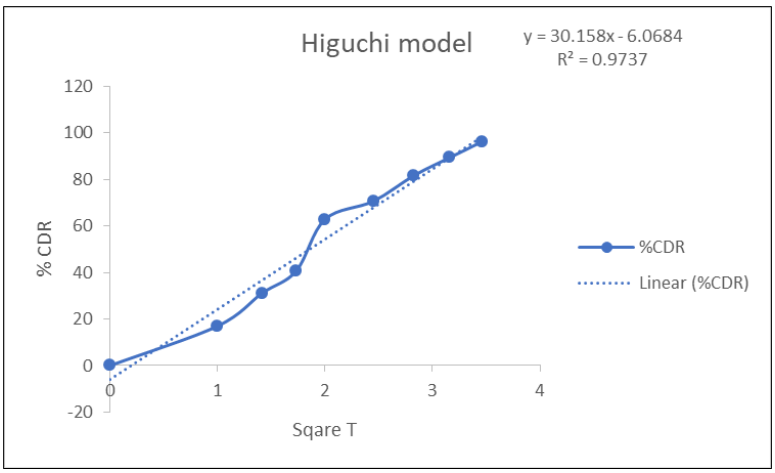


**Figure 7** Higuchi Model Optimized Formulation

*4.4.4. Korsmeyer Peppas*

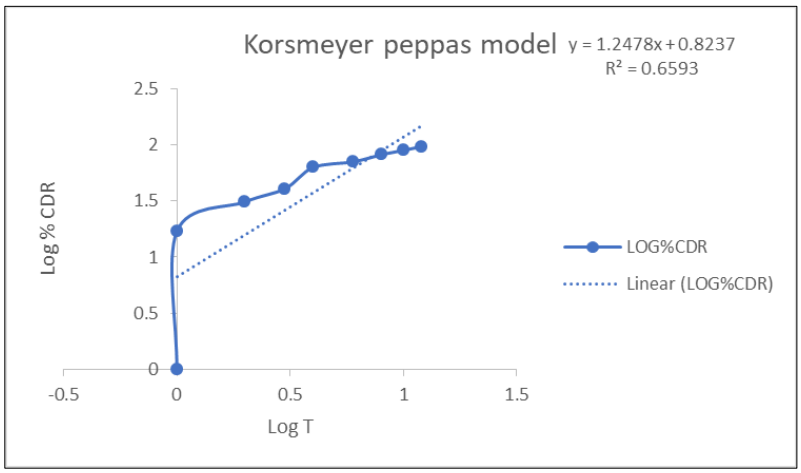


**Figure 8** Korsmeyer Peppas of Optimized Formulation

*4.4.5. Stability Studies*

There was no significant change in physical and chemical properties of the patches formulation F2- after 3 months. Parameters quantified at various time intervals were shown.

**Table 4** Results of Stability Studies of Optimized Formulation F-2

| F. Code | Parameters | Initial | 1st Month | 2ndMonth | 3rd Month | Limits as per Specifications |
|---|---|---|---|---|---|---|
| F-2 | 25$^0$C/60%RH % Release | 96.30 | 95.86 | 94.89 | 93.65 | Not less than 85 % |
| F-2 | 30$^0$C/75% RH % Release | 96.30 | 95.82 | 94.58 | 93.41 | Not less than 85 % |
| F-2 | 40$^0$C/75% RH % Release | 96.30 | 95.78 | 94.67 | 93.37 | Not less than 85 % |

## 5. Conclusion

Total 8 formulations were prepared in different ratios and evaluated drug entrapment efficiency and drug release studies. Among 8 formulation of Transferosomes, F2 formulation was maximum drug entrapment efficiency compared with other formulations. The optimized batch of Transferosomes was further incorporated into base and evaluated for Drug content and *In-vitr*o diffusion study. Transferosomes F2 formulation was optimized. The F8 formulation showed drug entrapment efficiency of 90.25 % and drug release of 96.30% sustained upto 12 hrs. Drug release followed zero order kinetics with fickian diffusion mechanism. Stability studies for optimized formulations were carried out at 25 ±0.5°C, 30 ±0.5°C and 40± 0.5ºCfor a period of 3 months. There was no significant variation found in physical appearance, average particle size and % drug content of the Transferosomes.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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