Improvement of solubility and dissolution of ebastine by fabricating phosphatidylcholine/ bile salt bilosomes

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Abstract: Although ebastine (EBT) can impede histamine-induced skin allergic reaction and persuade long acting selective H₁ receptor antagonist effects but its poor water solubility circumscribed its clinical application. The main objective of this research work was to improve the aqueous solubility and oral bioavailability of EBT by preparing EBT-loaded bilosomes (EBT-PC-SDC-BS). A thin film hydration method was used to prepare ebastine loaded bilosomes. The prepared-formulations were optimized considering size, morphology and entrapment efficiency. The SEM images revealed regular and spherical shape of bilosomes. Average size of the prepared EBT-PC-SDC-BS was 665.8 nm and zeta potential was around-32.9 mV with 89.05 % average entrapment efficiency (EE). Importantly, the solubility of EBT in water was amplified up to 17.9 μg/ml compared to pure drug (2 μg/mL) reflecting a highest solubility increase of 751 %. In vitro drug release results of prepared EBT-PC-SDC-BS exhibited improved release behavior. Finally, it is established from the results that the EBT-PC-SDC-BS could function as a favorable nano-carrier system to improve the solubility as well as dissolution of EBT.

Keywords: Bilosomes, ebastine, phosphatidylcholine, solubility, drug release.

INTRODUCTION

Ebastine (EBT) is a H₁ receptor inverse agonist with well-established therapeutic profile. It is used for common cold and different types of allergic diseases(Korfitis C et al., 2017). The powdery ebastine drug is reported with low solubility in water which is about 6.47e+04 mg/ml. EBT is insoluble in water practically. The available dosage forms of EBT in market show low bioavailability which may be linked to its poor aqueous solubility. Due to low solubility of EBT, it is included in Biopharmaceutics Classification System (BCS) class II. Thus, to get aspirational clinical benefits from EBT, the issue of drug solubility has to be addressed. The poor bioavailability of EBT is linked with metabolism and fast systemic elimination, when administrated orally, which has become the main hindrance in clinical application. The solubility, absorption, plasma concentration and anticipated pharmacological actions are very closely linked with each other. In order to improve the bioavailability of drug, consideration of these parameters while designing the dosage form are very essential (Georgaka et al., 2017).

Several approaches were tried in the past to increase the solubility and bioavailability of poorly soluble drugs. Previously, the solubility of ebastine was increased through tween 20, avicel and aerocil by using liquisolid technique (Harmalkar et al., 2019). Quasi emulsion solvent diffusion method was used to prepare the spherical crystal agglomerates of ebastine to enhance its solubility and dissolution (Hareeja MM and Al-Khedairy EB, 2018). Self-nanoemulsifying drug delivery system (SNEDDS) containing oleic acid, tween® 80, and ethanol was used to improve the solubility of ebastine (Kamisetti and Gupta 2017a). The solubility enhancement through bile acid salt and phosphatidylcholine has been successfully employed for different therapeutic agents like berberine (Jia et al., 2019), Androst-3β,5α,6β-triol (Guo et al., 2016).

Phosphatidylcholine (PC) are amphipathic molecules that orient themselves as cylindrical bilayers. The hydrophobic tails of the PC molecules are arranged toward inner side while hydrophilic head group are oriented outwards (Andoh et al., 2018). The amphiphilic property of PC makes it a good solubilizer for hydrophobic drugs (Jo et al., 2019). For that reason, PC is selected in the current work to enhance the solubility and dissolution rate of EBT. Bile salts are known to increase the apparent solubility of lipophilic drugs (El Naggar 2015). The dissolution of lipophilic drugs are increased with the concentration of bile salt (Amara et al., 2019). Bile salts also help to inhibit the crystallization of supersaturated solution of lipophilic drugs in biological
environment (Chen et al., 2015). The different types of disintegrants and hydrophilic excipients play critical role in the improvement of dissolution rate (Zhang et al., 2018). The combination of hydrophilic components with lipophilic ingredients are used to get fast dissolution rate for quick pharmacological results (Ditzinger et al., 2019). The presence of crosprovidone in the formulation is used to boost the dissolution rate (Pezzini et al., 2016).

The solubility and bioavailability of poorly water-soluble drugs could be enriched by using bile salt/PC bilosomes (Aburahma 2016). The performance of micellar system in drug delivery is highly appreciated by pharmaceutical researchers in current decades. The combination of bile salts and phospholipids have produced self-assembled structure in aqueous medium with the hydrophobic core and covered by bile salt (An et al., 2017). Both the ingredients are from natural sources and both are completely absorbed in the body, thus a biocompatible drug delivery system could be produced by their combination (McClements and Gumus 2016). The vesicles and micelles were intensively investigated for the delivery of hydrophilic and hydrophobic drugs (Hu et al., 2016). In this research work, the preeminence of bile salt/PC-bilosomes were used to enhance the solubility and bioactivity of EBT. Ebastine-loaded phospholipid-sodium deoxycholate bilosomes (EBT-PC-SDC-BS) could be good carrier system to enhance bioavailability of ebastine.

**MATERIALS AND METHODS**

**Materials**
Phospholipon™ 90H (Phosphatidylcholine) was gifted by Lipoid GmbH, Germany. Ebastine was purchased from Simz Pharmaceuticals, Pakistan. The Genome Pharmaceuticals, Pakistan, provided free sample of sodium deoxycholate (SDC). Crosprovidone (CP) was provided by Arsons Pharmaceuticals, Pakistan. All other lab reagents were used of analytical grade.

**Preparation of bilosomes (EBT-PC-SDC-BSs)**
Bilosomes were synthesized through a thin film hydration method with little modification (Lv et al., 2014) (Aburahma 2016). Briefly, the ebastine, phosphatidylcholine (PC) and bile salts were dissolved in ethanol in different proportion by mixing at ambient temperature as shown in the table 1. Then, round bottle flask containing above solution was attached to a rotary evaporator (Rotavapor R 300, Buchi Laborteknik AG) in order to remove organic solvent. The solution was evaporated at 60°C under reduced pressure until thin film was formed at the bottom of flask. The obtained thin film was hydrated with phosphate buffer pH 7.4 and subjected to ultrasound mixer for 15 minutes. Further size reduction was done by probe sonication. The suspension was centrifuged at 12000 rpm for 5 minutes and bilosomes were separated. The control bilosomes (without drug) were also prepared following the same method and procedure. The powder was obtained by freeze-drying method.

**Scanning electron Microscopy (SEM) and entrapment efficiency (EE %)**
The scanning electron microscopy was used to study the morphology of optimum EBT-PC-SDC-BSs. The optimized formulation was placed on grid and scanned under scanning electron microscopy (SEM).

**Particle size and zeta potential**
The size and zeta potential of EBT-PC-SDC-BSs were determined at room temperature by using MasterSizer 3000 (Malvern Analytical). Sample readings were taken in triplicate.

**Entrapment Efficiency**
The aqueous solution of EBT-PC-SDC-BSs was centrifuged at 15,000 rpm for 5 min to separate free and bilosomes incorporated drug. Then, aliquot was diluted with ethanol to interrupt the micellar structure to release drug. An isocratic liquid chromatograph (Shimadzu 10AD, Japan) with UV/Vis detector was used to quantify the entrapped ebastine in bilosomes. The C18 column (250 mm × 4.6 mm, particle size 5 μm) and mobile phase, methanol: water (80:20) were used in analysis. The flow rate of 1ml/min, column temperature 25°C and wavelength 262 nm were maintained. After filtration and proper dilution, solution was injected in HPLC to analyze the incorporated drug. (Kamisetti and Gupta 2017b). The entrapment efficiency was calculated by using following formula:

$$EE(\%) = \frac{Q_e}{Q_t} \times 100$$

Where $Q_e$ is the actual quantity of EBT entrapped in bilosomes and $Q_t$ is the total quantity of EBT used in preparing the bilosomes.

**Fourier transform infrared (FTIR) spectroscopy**
The EBT and bilosomes formulation were analyzed through Agilent Cary 360 FTIR using 4000 to 500 cm⁻¹ wavelength range (Jo et al., 2019).

**X-ray diffractometry (XRD)**
XRD studies of EBT and its bilosomes formulations was conducted at 30 kV and 35 mA using Bruker’s diffractometer. The samples were measured between 5 to 50° 20 ranges (Jo et al., 2019).

**Solubility studies**
The flask shake method was used to determine the solubility of ebastine in aqueous media. The excess quantity of EBT and prepared bilosomes were placed in separate glass tubes and 10ml of distilled water was added into each glass tube and placed in an orbital flask shaker (Heidolph, Germany) at 37°C for 36 hr. The samples were
collected, filtered and then tested at UV-spectrophotometer at 258 nm (Shimadzu, UV 2600 series, Japan).

**Fig. 1:** SEM images of (A) ebastine, (B) VB4 bilosomes.

**In vitro drug release**
The dissolution behavior of EBT and bilosomes was determined using USP apparatus II. The release behavior of EBT was determined using dialysis bag method. The release media was 0.1 N HCl (pH 1.2). EBT (10mg) was dissolved in propylene glycol to run as controlled and put into membrane bag by closing both side of membranes with cord clamps. The bilosomes equivalent to 10mg EBT were added in rest of the baskets for dissolution studies. The stirring speed was 100 rpm and the water bath temperature was maintained at 37 ± 0.5°C for all experiments. The predetermined volume of samples was withdrawn at predetermined time, filtered through 0.22 µm size filters and analyzed using high-performance liquid chromatography method (HPLC-10AD, Shimadzu, Japan). Samples were run in triplicate.

**Fig. 2:** FTIR of (A) ebastine, (B) sodium deoxycholate, (C) phosphatidylcholine, (D) VB1, (E) VB2, (F) VB3 and (G) VB4.

**Fig. 3:** PXRD of (A) pure drug, (B) VB1, (C) VB2, (D) VB3 and (E) VB4.

**Fig. 4:** Solubility of (A) pure drug, (B) VB1, (C) VB2, (D) VB3 and (E) VB4.

**STATISTICAL ANALYSIS**
The mean values were evaluated by one-way analysis of variance (ANOVA) using SPSS software (version 21). The value of \( p < 0.05 \) was considered significant.
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Table 1: Composition of bilosome formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Phosphatidylcholine %</th>
<th>Sodium deoxycholate %</th>
<th>Crospovidone %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB1</td>
<td>93</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>VB2</td>
<td>88</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>VB3</td>
<td>83</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>VB4</td>
<td>78</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2: Particle size, polydispersity index, zeta potential and entrapment efficiency of bilosomes formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Particle Size (nm), m.v± S.D</th>
<th>Polydispersity Index, m.v± S.D</th>
<th>Zeta Potential (mV), m.v± S.D</th>
<th>Entrapment Efficiency (%), m.v± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB1</td>
<td>677.2±0.61</td>
<td>0.18±0.01</td>
<td>27.1±0.06</td>
<td>93.8±0.05</td>
</tr>
<tr>
<td>VB2</td>
<td>663.1±0.70</td>
<td>0.11±0.01</td>
<td>32.4±0.23</td>
<td>90.6±0.11</td>
</tr>
<tr>
<td>VB3</td>
<td>662.4±0.81</td>
<td>0.086±0.02</td>
<td>34.8±0.46</td>
<td>87.6±0.23</td>
</tr>
<tr>
<td>VB4</td>
<td>660.7±0.09</td>
<td>0.09±0.03</td>
<td>37.6±0.35</td>
<td>84.2±0.05</td>
</tr>
</tbody>
</table>

RESULTS

Morphology, particle size, zeta potential and entrapment efficiency of bilosomes

The SEM scans revealed spherical shape of bilosomes with uniform distribution as shown in fig. 1. The average particle size, polydispersity index (PDI) and zeta potential of the optimal mixed micelles formulation were measured as 665.8±0.5 nm, 0.116±0.008, -32.9±0.95mV respectively. The details of particle size, PDI, zeta potential and entrapment efficiency are shown in table 2.

X-ray diffractometry (PXRD)

The diffractograms of EBT and bilosomes are presented in fig. 3. The major diffraction peaks of EBT were shown at 2θ angles 5°, 9°, 18.8°, 19.3° with high intensities. The intensities of diffraction were reduced in bilosomes formulations.

Solubility

The results of solubility are shown in the fig. 4. The maximum solubility was found in VB1. The concentration of phosphatidylcholine was higher in VB1 as compared to other formulations.

In vitro drug release

The dissolution profile of EBT-PC-SDC-BS showed slow release profile in 0.1 N HCl (pH 1.2) as shown in fig. 5 (P<0.05). The EBT pure drug exhibited about 20% drug release in 60 min. In comparison, the VB4 bilosomes showed prompt release in the first 30 min (P<0.05). This could probably due to the drug absorbed on the surface of bilosomes. VB-3 bilosomes released more than 80% of the drug within one hour compared to other formulations (P<0.05). VB-2 released drug at slower rate which is indication of strong and stable inner micellar core. VB1 bilosomes released less than 78% drug in one hour (P<0.05). The formulation VB4 provided fastest dissolution rate as about 88% drug was released in only 10 min.

DISCUSSION

The oral bioavailability of EBT is only 40 to 50% which is basically limited by its poor aqueous solubility. In our saturated solubility experiments, solubility of EBT was increased in phosphatidylcholine (PC) as the concentration of PC increased (Jo et al., 2019). The drug in the EBT-PC-SDC-BS remained intact while passing through harsh enzymes and acid rich GIT environment. EBT-loaded phosphatidylcholine and bile salt micelles have been evidenced to improve the aqueous solubility of drug. The strong interaction between PC and SDC
resulted into high drug loading and entrapment efficiency. The bilayer structure of PC was converted to micelles by the durable solubilization effect of bile salt (Guo et al., 2016). The concentration of bile salt also played critical role in increasing drug solubility and dissolution rate (Rajput and Chauhan 2017). At lower bile salt concentration, the obtained bilosomes were large in size. The concentration of bile salt greater than 30mM had significant effect on the size of vesicles (Arafat et al., 2017). The combined effect of PC and SDC on drug loading and solubility was greater as compared to alone SDC. The addition of disintegrant also helped in increasing the dissolution rate. The cros POVODIDE could create pores in the structure of bilosomes, ultimately, leading to increase in dissolution (Quodbach and Kleinebudde 2016). Furthermore, this lipophilic carrier system can transfer drug through lymphatic transport by bypassing the first pass effect of liver. The chances of lymphatic transport are increased by the presence of bile salt and phosphatidylcholine (PC).

The EBT-PC-SDC-BSs were found to be spherical and uniformly distributed in SEM scan. The monolayer formed by PC molecules were perforated by SDCs. The bile salt facilitated to form spherical structure. The medium size vesicles (500 to 700 nm) were obtained which might be due to the presence of crospovidone. The SDC surrounded the micelles producing negative surface charge on the micelles (Aburahma 2016). The negative charge value above -32mV was found indicating stable bilosomes. As, zeta potential above 30mV is considered suitable for the stability of colloidal dispersion.

The FTIR peaks in bilosomes formulations were completely matched with the EBT standard peaks reflecting no chemical interactions. The diffraction peaks of EBT were very prominent near 18.8°, 19.3° while very small peaks were appeared in VB1 and VB2 bilosomes. As the concentration of bile salts was increased, the peaks were almost disappeared in diffractogram. XRD analysis confirmed the reduction in crystallinity of EBT in prepared bilosomes. The in vitro drug release study that solubility and dissolution rate of EBT was significantly increased by incorporating into bilosomes. The drug release from the prepared bilosomes was fast as compared to pure drug (P<0.05). In addition, the bile salt due to its amphiphilic property improved the dissolution rate in the presence of disintegrant (Zarmpí et al., 2017). The required amount of SDC for solubilization needs special attention to be adjusted so that lowest proportion of phosphatidylcholine and bile salt could be incorporated in delivery system (Duan et al., 2015). Finally, the bilosomes system could enhance the solubility and bioavailability of EBT (O’Elia et al., 2019). The value of p obtained was less than 0.05 indicating that the results were statistically significant.

Future research plan and study limitation
The research activities were stopped due to Covid-19 pandemic. All scientific facilities were unavailable, which became the key constraint for further studies. In vitro permeability and in vivo bioavailability studies will be conducted for in depth exploration of bilosomes. In future, developed bilosomes will be incorporated in different dosage forms for bioavailability enhancement.

CONCLUSION
EBT-loaded PC-SDC-BSs formulations were successfully developed using thin film hydration method. The formulations were optimized based on size, shape, entrapment efficiency and in-vitro drug release. The prepared bilosomes profoundly increased the solubility and dissolution of ebastine. The phosphatidylcholine increased the solubility of EBT whereas the appropriate bile salt and disintegrant concentrations were found to be fundamental in accelerating the dissolution rate. The FTIR and XRD studies confirmed the development of bilosomes in amorphous form. In nutshell, the developed bilosomes could be a promising drug delivery system for improving the solubility as well as dissolution of EBT.

REFERENCES
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2306

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