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Solubility Enhancement of Ebastine by Self-Nanoemulsifying Delivery Strategy: Formulation, Optimization and Characterization

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ABSTRACT

The aim of the study was to develop and optimize Self-nanoemulsifying drug delivery systems (SNEDDS) for the improvement of solubility and dissolution of an anti-allergic drug, Ebastine, a BCS class II drug. Preliminary screening was carried out to select proper components combination of Oil (Oleic acid): Surfactant (Tween® 80): Co-solvent (Ethanol). Pseudo-ternary phase diagram experimental design was applied to formulate and optimize the SNEDDS containing 3:7 of (oil: S_{mix}). Drug-Excipient compatibility studies were performed by FTIR and found no chemical interaction between the drug and excipients. The systems were assessed for evaluation parameters like optical clarity in three stages by exposing the SNEDDS to heating-cooling cycle at 4 to 45 °C, centrifugation at 5000rpm and freeze-thaw cycles at -21 °C to 21 °C. The droplets of optimized SNEDDS formulation were found to be spherical with a size range of 76-111nm and emulsification efficiency of 97.67 ± 0.3% and 91.1 ± 0.06% drug release at the end of 30 minutes with a significant increase in dissolution rate compared to the marketed drug suspension under the same conditions. The optimized SNEDDS formulation charged for the accelerated stability studies at 40 °C/75% RH for three months revealed to be stable with 95.31 ± 1.4% drug content and 90.12 ± 1.98% drug release. It was hence concluded that the solubility of poorly soluble drugs like Ebastine can be effectively enhanced using Self nano emulsifying approaches with the use of Oleic acid, Tween 80 and Ethanol as Oil, surfactant and co-solvent respectively.

KEYWORDS: Self nano emulsifying drug delivery systems; Emulsification efficiency; Nano zeta sizer; Freeze-thaw cycles; Drug release; Stability.

Introduction

Lipid based formulations offer a potential platform for improving oral bioavailability of drugs especially those belonging to Biopharmaceutical Classification System (BCS) class II and class IV. Self-emulsifying systems use the concept of *in situ* formation of emulsion in the gastrointestinal tract. The mixture of oil, surfactant, co-surfactant, one or more hydrophilic solvents and co-solvent forms a transparent isotropic solution that is known as the self-emulsifying drug delivery system in the absence of external phase (water) and forms fine o/w emulsions or micro-emulsions spontaneously upon dilution by the aqueous phase in the GIT and is used for improving lipophilic drug dissolution and absorption. The ease of emulsification could be associated with the ease of water penetrating into the various liquids crystalline or gel phases formed on the surface of the droplet (O' Driscoll and Griffin, 2008). Other Potential advantages of these systems include enhanced oral bioavailability enabling reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug(s) towards specific absorption window in GIT, protection of drug(s) from the hostile environment in gut, control of delivery profiles, reduced variability including food effects.

According to Reiss, self-emulsification occurs when the entropy change, that favors dispersion, is greater than the energy required to increase the surface area of the dispersion. In addition, the free energy of a conventional emulsion formation is a direct function of the energy required to create a new surface between the two phases and can be described by equation

$$\Delta G = \sum_i N_i \pi r_i^2 \sigma$$

Where, G is the free energy associated with the process (ignoring the free energy of mixing), N is the number of droplets of radius, r, and s represents the interfacial energy (Reiss, 1975). With time, the two phases of the emulsion will tend to separate, in order to reduce the interfacial area, and subsequently, the free energy of the systems (Basalious et al., 2010). Therefore, the emulsions resulting from aqueous dilution are stabilized by conventional emulsifying agents, which form a monolayer around the emulsion droplets, and

hence, reduce the interfacial energy, as well as provide a barrier to coalescence (Singh et al., 2011; Shahnaz et al., 2011). Self-nano emulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water nanoemulsion when introduced into aqueous phases under gentle agitation (Elnaggar and El-Massik, 2009). SNEDDS spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification (Dixit and Nagarsenker, 2008).

Various techniques like Sonication, emulsion diffusion evaporation, multiple emulsion solvent evaporation (Trickler, 2008; Thomas et al., 2013; Anton et al., 2008; Mahmoud et al., 2009), Ultrasonication (Nermeen, 2014) etc, have been successfully employed in the formulation as self -nano emulsifying drug delivery systems. Ebastine is a long acting non-sedating selective peripheral histamine H1 receptor antagonist indicated for the treatment of seasonal and perennial rhinitis and Idiopathic chronic urticaria (Tagawa, et al., 2001). It is a BCS class II drug with a half-life of 15-19 hours. It is a basic compound that contains tertiary amine group with p^{ka}8.8 and partition coefficient of 7.64 (Maddens, et al, 2011). It does not become readily bioavailable when given orally (Van Cauwenberge et al., 2004; Simons, 2004). The potentiality of self-nanoemulsifying drug delivery systems have been successfully employed for various drugs like lacidipine and Adefovir dipivoxil (Basalious et al., 2010).

In this study, we made an attempt to enhance the solubility of Ebastine by self -nanoemulsifying approach using oleic acid as oil phase, tween 80 as surfactant and ethanol as solvent.

Materials and Methods

Ebastine was obtained as a gift sample from Vasudha Pharma Chem Pvt. Ltd, Hyderabad. Oleic acid, Tween 80 and Ethanol were procured from S.D. Fine Chemicals, Mumbai. All other reagents used were of analytical grade and used as provided.

Methodology

Solubility study of drug in various oils: The study was performed as described earlier with slight modifications (Suyang *et al.*, 2013). A preliminary solubility study of the drug was performed by adding an excess amount of drug in five mL of selected oils (Triacetin, Capmul GMO, Oleic acid, Castor oil, Arachis oil, Coconut oil) in 15mL capacity stoppered vials and shaken at 25±1.0°C for 72 hours. The tubes were centrifuged at 4000 rpm for 10 min and the supernatant was passed through a membrane filter (0.45 μm) to remove the un-dissolved drug. Solubility of Ebastine was determined by UV-Visible Spectrophotometer (LABINDIA UV+3000) by diluting appropriately with methanol at 254 nm. The results were shown in Table 1 and Figure 1.

Solubility of drug in Surfactants/Co-solvents: The solubility of Ebastine in various oils was determined by adding an excess amount of drug in five mL of selected surfactants/co-solvents (Tween 80, Tween 20, Span 60, Span 80, PEG 400, Ethanol) in 15mL capacity stoppered vials. The vials containing the mixtures were shaken at 25 ± 1.0°C for 72 hours to achieve solubility equilibrium. The tubes were centrifuged at 4000 rpm for 10 min and the supernatant was passed through a membrane filter (0.45 μm) to remove the undissolved drug. The samples were analyzed by UV-Visible Spectrophotometer (LABINDIA UV + 3000) after dilution with methanol at 254 nm. The results were shown in Table 2 and Figure 2.

TABLE 1

Solubility of drug in various oils.

Oily phase	Solubility [a] (mg/ml)
Oleic acid	210 ± 0.05
Capmul GMO	9 ± 0.15
Triacetin	17 ± 0.02
Castor oil	2 ± 0.03
Arachis oil	1 ± 0.5
Coconut oil	0.2 ± 0.1

[a]Data expressed as mean ± SD,n=3

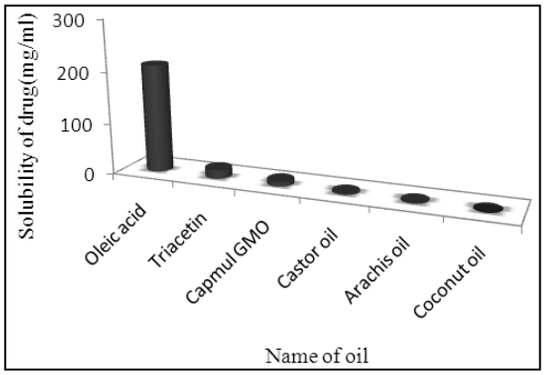

Fig. 1. Solubility profile of Ebastine in various oils.

TABLE 2

Solubility of drug in different surfactants/co-surfactants/co-solvents.

Surfactants	Solubility of drug [a](mg/ml)
Tween 80	38.40 ± 0.04
Tween 20	31.40 ± 0.02
Span 60	5 ± 0.05
Span 80	3 ± 0.08
PEG 400	60 ± 0.04
Ethanol	72.90 ± 1.10

[a]Data expressed as mean ± SD, n=3

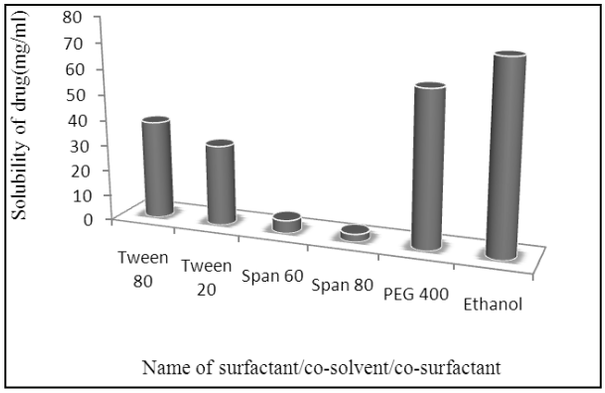

Fig. 2. Solubility studies of Ebastine in various surfactant and co-surfactants/co-solvents.

Construction of Pseudo Ternary phase diagram: Phase diagrams were constructed by selecting Oleic acid as oil phase. Tween80 as surfactant; and Ethanol as co-solvent from the results obtained from solubility studies. Surfactant and co-surfactant (S_{mix}) were mixed in different ratios (1:1, 2:1 and 3:1) to produce three formulations of the system. These S_{mix} were chosen in increasing concentration of surfactant with respect to co-surfactant for detailed study of phase diagram for the formulation of nano-emulsion of Ebastine. Mixture of the oil and S_{mix} were prepared at ratios (w/w) of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7; 2:8, 1:9 and 0:10 in vials. Pseudo ternary phase diagrams of oil phase, S_{mix} and aqueous phase were developed using the aqueous titration method. To the resultant mixture, distilled water was added drop wise and observed for transparency. The phase diagrams were constructed representing aqueous, oil and S_{mix} phases at fixed ratios using **CHEMIX** software as shown in Figure 3 to 6.

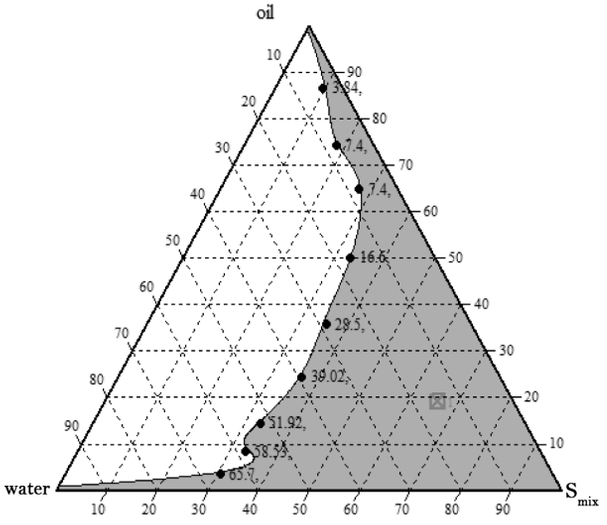

Fig. 3. Surfactant & Co-surfactant mix 1:1 Ratio.

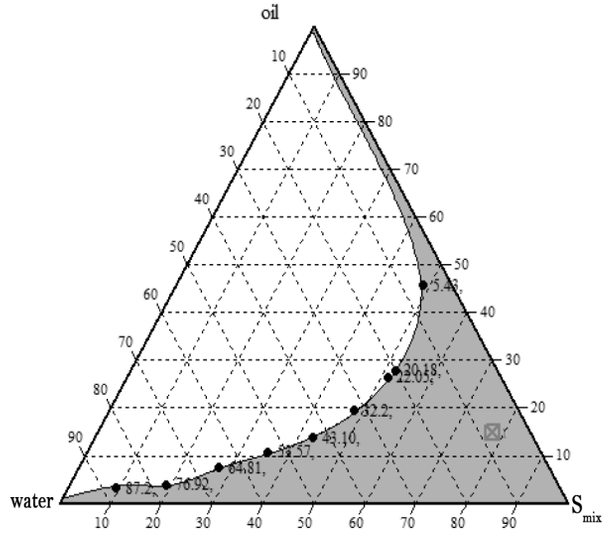

Fig 4. Surfactant & Co-surfactant mix 2:1 ratio.

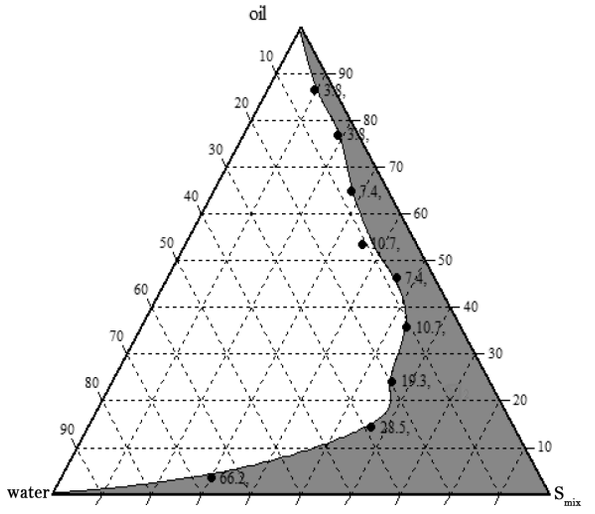

Fig. 5. Surfactant & Co-surfactant mix 3:1 ratio.

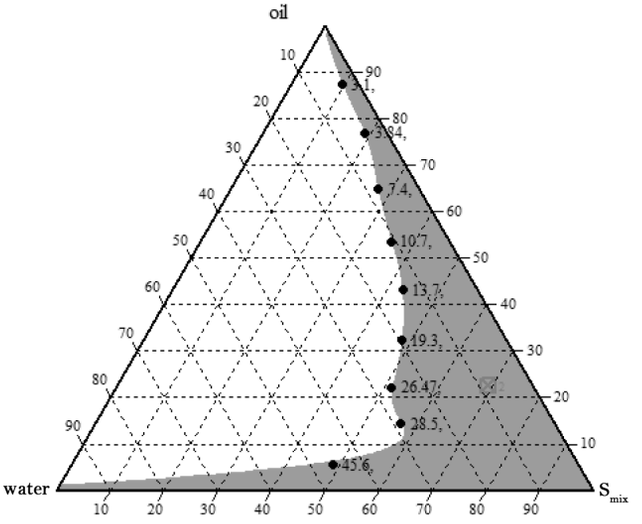

Fig. 6. Surfactant & Co-surfactant mix 1:2 ratio.

Formulation Design of SNEDDS Containing Ebastine

From the pseudo ternary phase diagrams, the formulations containing oil phase in which the drug got solubilized completely and which could accommodate the optimum quantity of S_{mix} and distilled water were selected for the study. Subsequent to the identification of emulsion region in the phase diagrams, the emulsion formulations were selected at desired component ratios. All the ratios in this study were reported as weight-to-weight ratios (W/W). The ratio of S/CS (Smix) 1:1 was used for preparation of SNEDDS. Formulations were selected from the nano-emulsion region of the constructed phase diagram to incorporate drug into the oil phase. The formulations were categorized with Oil: S_{mix} ratios of 2:8, 3:7, 4:6, 5:5, 6:4, and 7:3. The formulations were prepared by Ultra-sonication method initially dissolving the drug in oil phase (Oleic acid) at 45ºC in an isothermal water bath for 10 min. Surfactant (Tween 80) was then added and the mixture was cooled

to ambient temperature to which co-surfactant (Ethanol) was added. The mixture was sonicated for 30 min until a clear solution was obtained. The formulation was equilibrated at ambient temperature for atleast 48 hours and examined for signs of turbidity (or) phase separation. The formulations were shown in Table 3.

TABLE 3

Composition of SNEDDS Formulations.

Formulation code	Ebastine (mg)	Oleic acid % (w/w)	Tween 80 + Ethanol % (w/w) 1:1 ratio of Smix
F1	10	20	80
F2	10	30	70
F3	10	40	60
F4	10	50	50
F5	10	60	40
F6	10	70	30

Characterization of SNEDDS Formulations

Dispersibility test: The efficiency of self-emulsification of the Nano emulsion was assessed using a standard USP dissolution apparatus type II. One mL of each formulation was added to 500 mL of water at 37 ± 0.5°C at 50 rpm. The *in vitro* performance of the formulations was visually assessed using the following grading system as shown in Table 4. Results of self-emulsification and spontaneity were observed as shown in Table 5.

TABLE 4

Grades of self-emulsification.

Grade	Dispensability and appearance	Time of Self emulsification (min)
A	Rapidly forming nano emulsion, having a clear or bluish appearance	<1
B	Rapidly forming, slightly less clear emulsion, having a bluish white appearance	<2
C	Fine milky emulsion	<2
D	Dull, grayish white emulsion having slightly oily appearance that is slow to0020emulsify	<3
E	Formulation, exhibiting either poor/ minimal emulsification with large oil globules present on the surface.	<3

TABLE 5

Assessment of physical compatibility by Visual observation of prepared batches.

S. NO.	Formulation	Self-emulsification time [a](sec)
1	F1	7.5 ± 0.04
2	F2	9.1 ± 0.09
3	F3	10 ± 0.12
4	F4	12 ± 0.02
5	F5	14 ± 0.05
5	F6	15 ± 0.01

[a]Data expressed as mean ± SD, n=3

Thermodynamic Stability Studies

Heating-cooling cycle: Ebastine SNEDDS filled in hard gelatin capsules were stored alternatively at 4 ºC and at 45 ºC. The capsules were stored for 48 h at each temperature and repeated to complete six cycles. The capsules that withstand the heating cooling cycle were subjected to centrifugation test.

Centrifugation test: The selected capsules were centrifuged at 5000 rpm for 30 minutes and observed for any sign of phase separation, creaming or cracking. The capsules showed maximum stability were selected for freeze-thaw cycles.

Freeze-thaw cycles: Capsules passed the centrifugation test were exposed at -21 ºC and 21 ºC. Capsules were stored at each temperature for not less than 24h and the capsules found to endure the harsh conditions of the temperature changes were selected for further evaluation studies. The results were shown in Table 6.

Drug-Excipient Compatibility Studies

FTIR spectra of Ebastine and nano emulsion formulation were obtained by means of a FTIR spectrophotometer (bruker-alpha T). The samples measurements were attempted with the accumulations of 20 scans and a resolution of 4 cm^{-1} over the range of 400-4000 cm^{-1}. After running the spectra, significant peaks relating to major functional groups were identified; spectra of the subsequent samples of the same compound were compared with the original. The results were shown in Figure 7 and 8 & Table 7.

TABLE 6

Thermodynamic stability and Dispersibility test of different formulations.

Formulation code	Effect of Temperature on Phase Separation, Flocculation, Precipitation						Dispersibility	Study Inference
	After 4 week		After 8 week		After 12 week			
	2-8 ºc	RT	2-8 ºc	RT	2-8 ºc	RT		
F1-F6	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Grade A	Pass
	Centrifugation stability data (Phase Separation)							
	After 1 month		After 2 month		After 3 month			
F1-F6	No Phase separation will occurs.						Grade A	Pass
	Heating and cooling cycle(Creaming or Cracking)							
	After 12 hr		After 24 hr		After 48 hr			
F1-F6	No Creaming/Cracking occurs.						Grade A	Pass
	Freeze thaw cycle (Phase Separation)							
	at -21 °C		at 5 °C		at 25°C			
F1-F6	No Phase separation will occurs.						Grade A	Pass

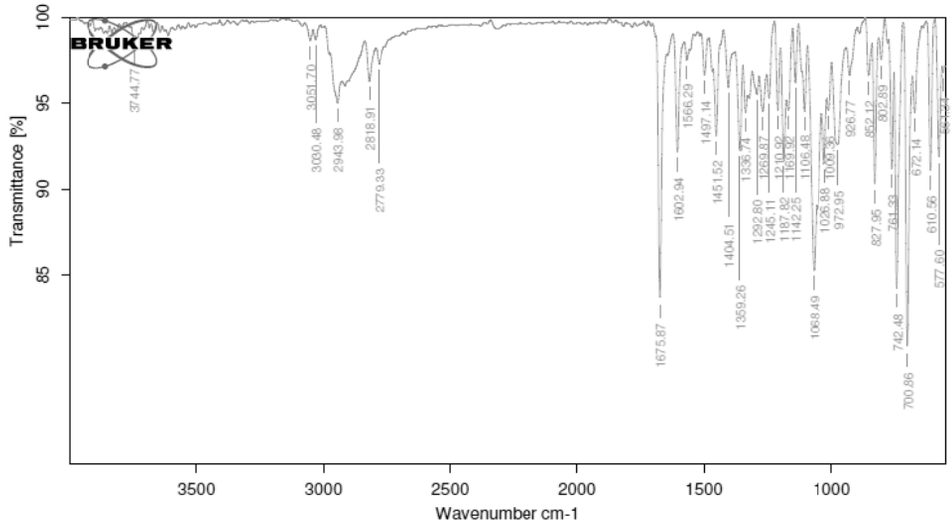

Fig. 7. FT-IR Spectrum of Ebastine.

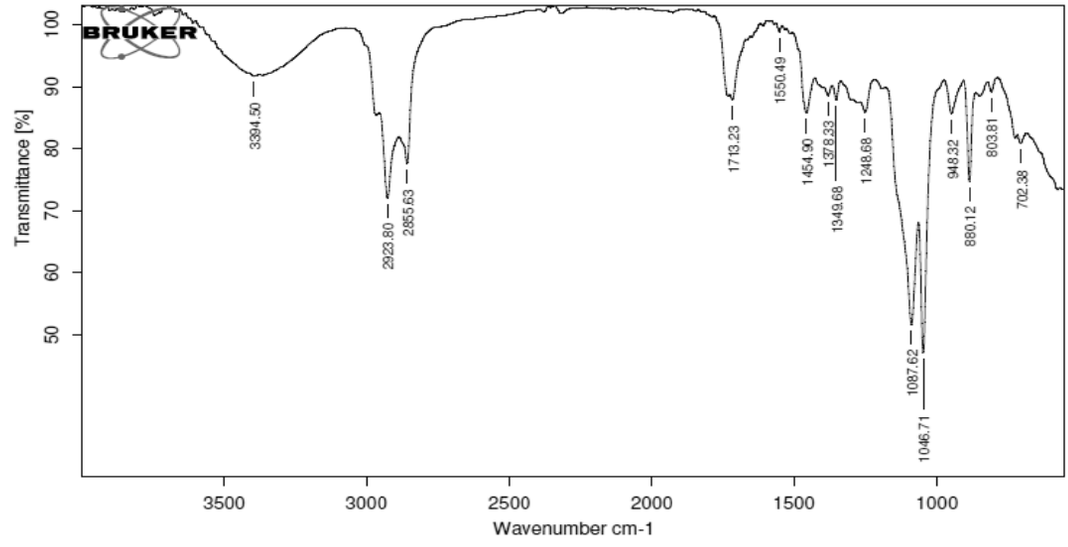

Fig. 8. FT-IR Spectrum of physical mixture Ebastine and Oleic acid, Tween 80 & Ethanol.

TABLE 7

Characteristic peak of Ebastine in FT-IR spectrum.

Standard (cm^{-1})	Band obtained in the drug (cm^{-1})	Band obtained in the mixture of drug with all excipients (cm^{-1})	Functional group (cm^{-1})
1500-1400	1451.5	1454.9	C-C stretching(in ring)
1870-1550	1675.6	1713.2	C=0 stretching
3000-2850	2923.8	2943.9	C-H stretching(alkanes)
1250-1020	1068.4	1045.7	C-N aliphatic amines
1335-1250	1245.1	1248.6	C-N aromatic amines

Droplet size Determination

The droplet size of the emulsions was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of theparticles) using Nano Zeta sizer (Horiba Instruments, Japan) able to measure sizes between 10-3000 nm. The formulation (0.1 ml) was dispersed into 100 ml of water under gentle stirring in a glass beaker. Then a 1 mL aliquot was withdrawn and added into a sample cell for droplet size measurement. Light scattering was monitored at 25°C at angle 90°. The dispersed formulations were measured after dilution (1:10000) with distilled water to produce the required count rate (50-200) to enable accurate measurement. Each size value reported was the average of at least three independent measurements. The results were shown in Table 8 and Figure 9.

TABLE 8

Droplet size measurements of all formulations.

Formulation	Mean droplet size *(nm)	Poly dispersity index	Zeta potential
F1	95 ± 0.02	0.332	-22.3 mv
F2	76 ± 0.01	0.311	-26.2 mv
F3	89 ± 0.03	0.341	-21.4 mv
F4	92 ± 0.02	0.328	-23.2 mv
F5	109 ± 0.03	0.338	-19.5 mv
F6	111 ± 0.01	0.359	-18.6 mv

*Values are mean ± SD; n = 3

TABLE 9

Drug encapsulation capacity of all L-SNEDDS formulation.

Formulations	Drug loading efficiency [a] (%)
F1	89.42 ± 0.2
F2	97.67 ± 0.3
F3	90.53 ± 0.1
F4	92.22 ± 0.2
F5	88.33 ± 0.4
F6	84.26 ± 0.5

[a]Data expressed as mean ± SD, n = 3

Zeta Potential

Zeta potential is used to identify the charge of the droplets. The magnitude of zeta potential was an indication about stability of colloidal system. Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment. Zeta potential measurements were carried out on the same diluted sample using the equipment and operating conditions like Nano Zeta sizer (Horiba Instruments, Japan at Light scattering was monitored at 25 ºC at a 90º angle, and the zeta potential values were calculated according to the Smoluchowski equation. A zeta potential value of ± 20 mV is sufficient for stability. Results were indicated in Table 8 and Figure 10.

Poly dispersity index

Uniformity of globule size in SNEDDS formulation was analyzed by the fluctuations in light scattering due to Brownian motion of the particles) using Nano Zeta sizer (Horiba Instruments, Japan) at 25 ºC and a scattering angle of 90 ºC. Results were indicated in Table 8.

Viscosity: Viscosity studies are necessary for SNEDDS to characterize the system physically and to control its stability determined by Brookfield Viscometer (Japan) DV-E using spindle RV-6 at 100 rpm at 25 ± 0.5 ºC.

Scanning electron microscopy (SEM): The formulation with least mean particle size among the six formulations was analyzed for scanning electron microscopy to confirm the particle size. From the results of particle size analysis F2 was selected and analyzed for SEM the results of the analysis also confirms that the formulation shows least particle size that is less than 100nm which was within the required limit (20nm-200nm). The results were shown in Figure 9.

Robustness to Dilution

The effect of dilution was evaluated by diluting 50mg of SNEDDS to 50 mL with various dissolution media viz. water, phosphate buffers (pH 1.2, 6.8 & 7.4). The diluted formulations were stored and observed after 12h for any signs of phase separation or drug precipitation.

Drug Loading Efficiency

The percentage of drug entrapped in globules was determined by mixing 50 mg of formulation with methanol to make up the volume up to 50 mL on magnetic stirrer for 8h. Supernatant was filtered and analyzed Spectrophotometrically at 254 nm.

Drug loading efficiency =

$$\frac{\text{Initial drug load} - \text{Amount of drug in filtrate}}{\text{Initial drug load}} \times 100$$

and the results were tabulated as shown in Table 9.

In vitro Release Studies

The release of Ebastine from the SNEDDS formulation was determined according to USP dissolution apparatus type-II. The SNEDDS filled in hard gelatin capsules were placed in 900 mL of 0.1N HCl and agitated at 50rpm at 37 ± 0.5 ºC. At predetermined time intervals, 5 mL of the samples were withdrawn, filtered through 0.45µm membrane filters and the drug concentration was determined at 209 nm. The volume removed was replaced each time with fresh dissolution medium to maintain sink conditions. Cumulated released amounts were plotted as a function of time. Table 10 and Figure 10 shows cumulative percentage drug release of all formulations.

TABLE 10

Cumulative percentage drug release of all formulations.

Time (min)	% Cumulative Percentage drug release *					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	45.2 ± 0.03	69.2 ± 0.02	46.5 ± 0.03	54.3 ± 0.01	43.2 ± 0.03	38.3 ± 0.02
10	49.8 ± 0.01	75.3 ± 0.01	52.7 ± 0.01	62.5 ± 0.04	49.4 ± 0.02	42.8 ± 0.01
15	58.6 ± 0.02	82.2 ± 0.02	59.2 ± 0.02	71.8 ± 0.05	55.2 ± 0.01	48.6 ± 0.03
20	63.2 ± 0.05	88.3 ± 0.03	68.9 ± 0.08	74.2 ± 0.05	63.8 ± 0.06	51.2 ± 0.04
30	70.2 ± 0.09	91.1 ± 0.06	77.3 ± 0.09	82.8 ± 0.01	68.2 ± 0.02	62.9 ± 0.02

*Values are mean ± SD; standard deviation (n=3)

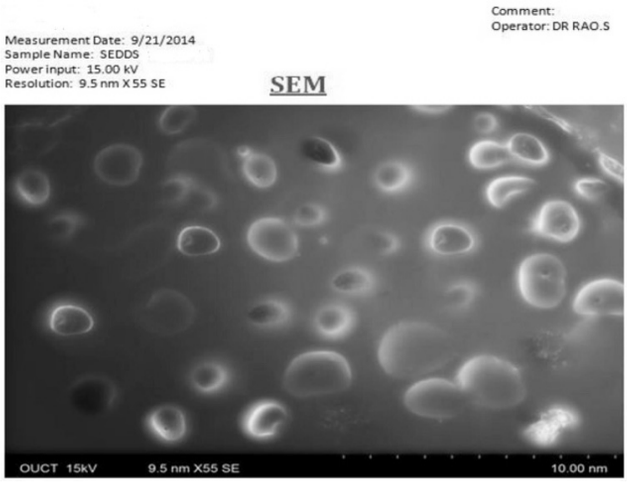

Fig. 9. The scanning electron microscopic study on the external morphology of the SNEDDS F2 formulation.

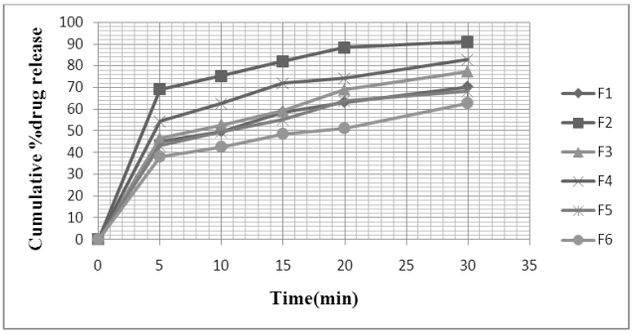

Fig. 10. Dissolution profile of SNEDDS containing ebastine.

Comparison with Marketed Product

The prepared promising batch F2 of SNEEDS was compared with marketed product (10 mg) and pure drug (10 mg) with respect to drug dissolution profile. The results were shown in Table 11.

Accelerated Stability Studies of Ebastine SNEDDS

Stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and also enables recommended storage conditions. The optimized Ebastine loaded SNEDDS was stable when stored at 40°C ± 2°C/75% ± 5% RH for three months. The results were shown in Table 12.

TABLE 11

Comparative dissolution profile of promising batch with marketed product and plain drug.

Time (Min)	Cumulative % drug release*		
	F2	Mkt	Drug
0	0	0	0
5	3.1 ± 0.352	28.6 ± 0.15	69.2 ± 0.02
10	5.7 ± 0.365	35.2 ± 0.219	75.3 ± 0.01
15	6.9 ± 0.412	39.2 ± 0.345	82.2 ± 0.02
20	8.1 ± 0.428	40.3 ± 0.245	88.3 ± 0.03
30	11.3 ± 0.496	43.8 ± 0.321	91.1 ± 0.06

*Values are mean ± SD; standard deviation (n=3)

TABLE 12

Stability study of Ebastine SNEDDS at 40°C ± 2°C /75% ± 5% RH.

Time (days)	Drug content [a] (%)	Drug release [a] (%)
0	97.67 ± 0.3	91.1 ± 0.06
30	96.45 ± 0.4	90.84 ± 1.1
60	96.12 ± 1.2	90.51 ± 1.9
90	95.31 ± 1.4	90.12 ± 1.98

[a]Data expressed as mean ± SD, n = 3

Results and Discussion

Solubility study of drug in various oils: The results from Fig. 1 and Table 1 showed that Oleic acid was found to solubilize the maximum amount of the drug 210 ± 0.05 mg/mL.

Solubility of drug in Surfactants/Co-solvents: The results showed that Tween80® was found to solubilize the maximum amount of the drug (38.40 ± 0.04 mg/mL). Ethanol was found to solubilze the maximum amount of the drug (72.90 ± 1.10 mg/mL) as shown in Figure 2 and Table 2.

Figure 3 represents Pseudo-ternary diagrams of 1:1 nanoemulsion region in blue colour. It was observed that when co-surfactant was added along with surfactant, the interfacial film became more fluid and no liquid crystalline area was found in the phase diagram. A large o/w nano emulsion area was observed.

As the surfactant concentration was increased in S_{mix}1:1, a higher nano emulsion region was observed. It may be due to further reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. There may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers.

Figure 4 represent the ratio of surfactant to co-surfactant to be increased. With further increase in surfactant concentration in S_{mix} 1:1 to 2:1, the nano emulsion region decreased as compared to 1:1. The ratio of surfactant concentration was increased from 1:1 to 3:1 compared to co-surfactant, the nanoemulsion area decreased shows in Figure 5. The ratio of co-surfactant concentration was increased from 1:1 to 1:2 compared to surfactant, the nanoemulsion area decreased shows in Fig. 6.

Characterization and Evaluation of SNEDDS

Dispersibility test: Self-emulsification time of all formulations as the concentration of surfactant increases, the spontaneity of emulsification process increased as shown in Table 6. This may be due to capacity of Tween 80® in reducing the interfacial tension and that the co-solvent further lower the interfacial tension between O/W interface and also influenced interfacial film curvature, may show impact on spontaneous emulsification process.

Thermodynamic Stability Studies of Ebastine SNEDDS

Thermodynamic stability studies

Heating-cooling cycle: Ebastine SNEDDS filled in hard gelatin capsules were stored alternatively at 4^0C

and at 45 ºC. The capsules were stored for 48 h at each temperature and repeated to complete six cycles. The capsules that withstand the heating cooling cycle were subjected to centrifugation test.

Centrifugation test: The selected capsules were centrifuged at 5000 rpm for 30minutes and observed for any sign of phase separation, creaming or cracking. The capsules showed maximum stability were selected for freeze-thaw cycles.

Freeze-thaw cycles: Capsules passed the centrifugation test were exposed at -21 ºC and 21 ºC. Capsules were stored at each temperature for not less than 24h and the capsules found to endure the harsh conditions of the temperature changes were selected for further evaluation studies.The results were shown in Table 6.

Drug-Excipient Compatibility Studies

FTIR spectra revealed no possible chemical interactions between the drug and the excipients as shown in figure 7 and 8 and Table 7.

Droplet Size Determination

Droplet size distribution following self-nano-emulsification is a critical factor to evaluate a self-nanoemulsion system. Droplet size was an important factor in self emulsification performance because it determines the rate and extent of drug release, as well as stability of the emulsion. An increase in the ratio of the oily phase (Oleic acid) resulted in a proportional increase in particle size because of the simultaneous decrease in the s/cs proportion. Range of globule sizes of SNEDDS were 20-200nm. Globule size of Oleic acid SNEDDS formulation is found to be decreased with reduction in oil content. The S_{mix} : Oil ratio was 7:3, the droplet size formed was smaller in comparison with 8:2, 9:1, 5:5 and 4:6 ratio of S_{mix} : Oil were shown in Table 8 and Figure 9.

Zeta Potential

Zeta potential used to identify the charge of the droplets. The optimized SNEDDS F2 formulation shows high absolute zeta potential value of -26.2 mV in Fig. 10. Increased absorption of SNEDDS can also be assessed by the charge of the oil droplets which is usually found to be negative due to the presence of free fatty acids. The emulsion stability is directly related to the magnitude of the surface charge. Generally, an increase of electrostatic repulsive forces between nanoemulsion droplets prevents the coalescence of droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. The magnitude of zeta potential was an indication about stability of colloidal system. Hence, the optimized SNEDDS (F2) would not exhibit threshold agglomeration as the nanoemulsion was stabilized by a greater zeta potential (negative) and steric stabilization effect.

Poly Dispersity Index

The poly dispersity index of optimized formulation F2 shows 0.311. Poly dispersity index (PI) is a measure of particle homogeneity and it varies from 0.0 to 1.0. The poly dispersity index below 0.4 indicates uniformity in the droplet size distribution after dilution. More closer is the value to zero; the more homogenous are the particles. When compared to all formulations optimized F2 formulation shows good uniformity in the droplet size distribution after dilution with water and the results were shown in Table 8.

Viscosity Determination

From viscosity determination, it was observed that as the concentration of oil increased viscosity of formulations decreased. The viscosity of formulation F2 was found to be 27.33 ± 1.15cps.The viscosity of the Ebastine SNEDDS is crucial in determining its ability to be filled in hard or soft gelatin capsules. If the system has very low viscosity, it may enhance the probability of leakage from the capsule and the system with very high viscosity may create problem in pourability. The viscosity value of formulation <10,000 cps, is generally considered as suitable for developed SNEDDS which can be filled in hard gelatin capsules by commercial liquid filling equipment's. The viscosity values are also known to provide a linking on whether the system is w/o or o/w type.

Scanning Electron Microscopy

It was evident from Fig. 11 that the SNEDDS formulation F2 had spherical, discrete, and non aggregated globules with globule size distribution within range of 20-200 nm. The surface morphology, spherical nature, smoothness, formation of aggregates and the size distribution of nanoemulsion was clearly seen.

Robustness to Dilution

The diluted SNEDDS formulation of Ebastine (F2) with 250 mL of each dispersion media showed no visible signs of phase separation or drug precipitation after storage for 12 h at 37 + 0.5 ºC.

Drug Loading Efficiency

Table 9 showed that Formulation F2 (Oil 30% w/w and S_{mix} 70% w/w) showed maximum encapsulation efficiency of 97.67 ±0.3%.

From all six formulations, F2 and F4 shows satisfactory result in drug release when compared to other formulations. The formulation F2 (Oil 30% w/w and S_{mix} 70% w/w) showed highest release rate among all the SNEDDS formulations i.e. 91.1 ± 0.06% at 30 min as shown Table 10 and in Figure 12. Thus, it was considered as the optimized SNEDDS formulation.

A comparative dissolution profile studied for pure drug, optimized formulation and marketed formulation was shown in Table 11 which shows that F2 shows better release than marketed tablet.

Accelerated Stability Studies of Ebastine SNEDDS

It was observed from Table 12 that no apparent change in the physical parameters such as homogeneity and clarity, reflecting the excellent stability of the developed SNEDDS formulation. Furthermore, there was

no significant difference in the drug content, and *in vitro* dissolution profile.

Conclusions

It can be thus concluded that, self nano emulsifying drug delivery systems can be successfully employed as a novel and commercially feasible technique to enhance the solubility of poorly water soluble drugs like Ebastine.

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