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Formulation, characterization, and *in vitro–in vivo* evaluation of self microemulsifying drug delivery system of Ebastine by spray drying technology using solid carriers

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ABSTRACT

Aim: The present work was aimed at formulating novel liquid self microemulsifying oil formulation and its solid form by spray drying method for improving the solubility of Ebastine, an anti-allergic drug and BCS class II drug, evaluating its in vitro and in vivo potential. **Results:** The self emulsifying oil formulation was comprised of Rice Bran Oil, Tween 40, Capryol 90. Pseudoternary phase diagrams were used to evaluate the micro emulsification existence area, and the release rate of Ebastine was investigated using an in vitro dissolution test. Accelerated stability testing of the formulation was also assessed at 400C/75% RH for 3 months and the results showed that it was stable with respect to drug content and visual inspection. Group of mice were sensitized with intra peritoneal injections of ovalbumin. Starting from day 21, nasal symptoms were evaluated after mice were challenged with intranasal OVA administration. By counting the unit time of nasal rubbing and number of sneezing events for the duration of 10 minutes and the evaluation was carried out continuously for 10 days. Ebastine formulation was demonstrated to have antiallergic effects on the nasal symptoms of OVA induced mouse model. **Conclusion:** Ebastine formulation has the therapeutics potential for the treatment of allergic Rhinitis. The solid self emulsifying oil formulation is a promising dosage form for poorly water soluble and low bioavailable Ebastine.

Keywords: SMEDDS, spray drying, poorly soluble drug, bioavailability

INTRODUCTION

Most of the new drug candidates have severe problems of poor water solubility and oral delivery of such candidates leads to low bioavailability.[1] The limited dissolution rate arising from low solubility frequently results in the low bioavailability of orally administered drugs and compounds with aqueous solubility lower than 100 μg/mL generally leads limited absorption.[2]

Oral route is the major route of drug delivery for the chronic treatment of many diseases. However, oral delivery of 50% of the drug compounds is hampered because of the highly lipophilic nature of the drug candidate. Nearly 40% of new chemical entities exhibit low solubility in water, which leads to poor oral bioavailability, high intra- and inter-subject variability, and lack of dose proportionality. Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution. Modification of the physicochemical properties, such as salt formation and particle size reduction of the substance may be approaches to improve the dissolution rate of the drug. However, these methods have their own limitations and problems. For instance, the weakly acidic and basic drugs may leads to neutralization at pH 7; therefore, salt formation of neutral compounds is not feasible and the synthesis of weak acid and weak base salts may not always be practical. Moreover, the salts that are formed may convert back to their original acid or base forms and lead to aggregation in the GI tract. Particle size reduction may not be desirable in situations where handling difficulties and poor wettability are experienced for very fine powdered substances.[3]

The availability of the drug for absorption can be enhanced by presentation of the drug as a colloidal dispersion system. A potential strategy to improve the oral bioavailability of poorly water-soluble drug molecules and substances is the

lipid- and surfactant-based drug delivery systems, covering a large array of different drug delivery systems from oil solutions/suspensions, emulsions, micellar systems to self-micro-emulsifying drug delivery systems (SMEDDS), i.e., self-emulsifying oil formulations.[4]

Self-emulsifying drug delivery systems (SEDDS) formulations are defined as isotropic mixtures of oils, surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/co-surfactants. On mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can be formed into fine oil in water (o/w) emulsions. Self-emulsifying formulations spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation (peristaltic movement) necessary for self-emulsification. When compared with emulsions, which are sensitive and metastable dispersed forms, self-emulsifying oil formulations are physically stable formulations that are easy to manufacture. For lipophilic drug, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood time profiles.[5,6] The spray drying method is one of the best methods for the solidification of SEDDS/SMEDDS. Spray-dried products exhibit more attractive properties such as reduced particle size, increased dissolution, and improved flow properties than their pure forms and broaden their application range.[7,8] Spray drying involves pumping a liquid feed through a atomization device, which forms small droplets that are sprayed into hot air stream to facilitate rapid drying and producing a fine powdered product.[9,10] It is a continuous processing operation involving a combination of several stages, namely atomization, mixing of spray with the hot air, evaporation, and product separation.[11] Physical properties of the spray-dried powder are very important and mainly depend on various operating parameters such as air flow and temperature, nozzle type, nozzle pressure, feed solution properties, and feed flow. The concentration of feed solution materials used during spray drying have a considerable effect on the reconstitution, particle size, particle surface, and so on.[12] According to the size range of their oil droplets, self-emulsifying oil formulation form microemulsion ranging in droplet size from 100 to 250 nm. Finer microemulsion of <100 nm can be obtained using SNEDDS. In this study, we made an attempt to enhance the solubility of Ebastine by self-microemulsifying approach using Rice bran oil as oil phase, Tween 40 as surfactant and capryol 90 as cosurfactant.[13]

MATERIALS AND METHODS

Chemicals and Reagents

Ebastine was gifted from Micro Labs, Bengaluru. Labrafil 2125 CS, Labrasol, Maisine 35-1, Transcutol HS were gifted by Gattefosse (Saint Priest Cedex, France). Capmul MCM EP, Capmul MCM NF, Capmul PG-8NF, Captex 300 EP/NF, Captex 200 P were gifted by ABITEC Corporation (USA). Tween 20, Tween 80, Span 20, Span 80, propylene glycol, polyethylene glycol (PEG) 200, PEG 400 were purchased from SD Fine (Mumbai, India). Aerosil 200 was purchased from Fine Chem Industries (Mumbai, India). Acetonitrile was purchased from Fischer Scientific Co. (India). All the other chemicals and reagents used were of analytical grade.

Animals

Six-week-old female mice (Swiss albino) were used for the antiallergic and antihistaminic activity. The animals were maintained at 12-h light/dark cycle, temperature (24°C–25°C), and humidity (60%) and were supplied with food and water *ad libitum*. The animal requirement was approved by the Institute Animal Ethics Committee, and all experiments were conducted as per the norms of the committee for the purpose of supervision of experiments on Animals, India.

Solubility Studies

Solubility of Ebastine was studied in different solvents like distilled water, 0.1N HCl, methanol, methylene chloride by using the shake flask method. The self-micro emulsifying formulation consists of oil, surfactant, co-surfactant and drug should be a clear liquid at ambient temperature when introduced to aqueous phase and should have good solvent

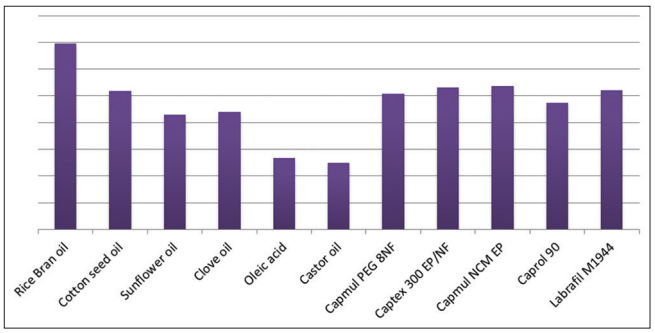

Figure 1: Solubility of Ebastine in oils

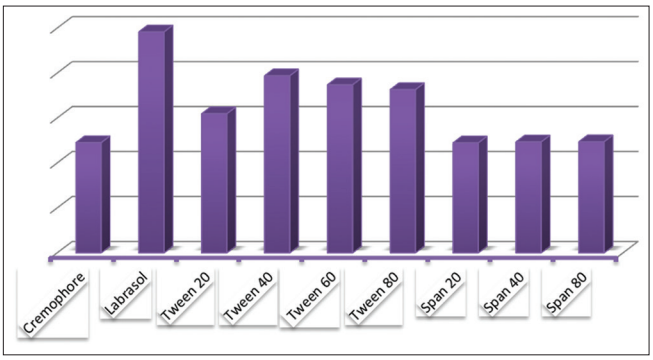

Figure 2: Solubility of Ebastine in surfactants

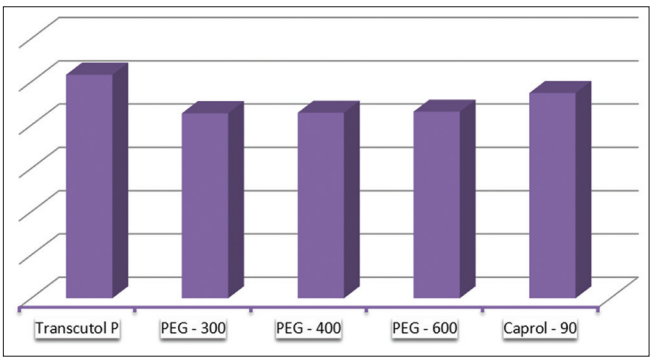

Figure 3: Solubility of Ebastine in co-surfactants

properties to allow the presentation of the drug in solution. In self-emulsifying system drugs are solubilized in oily core and/or in the interface of the emulsion structure. Hydrophobicity of the drugs and presence of surfactant, co-surfactant, and oil affect the solubility.

The solubility of Ebastine in different oil, surfactant, and cosurfactant are shown in Figures 1-3 respectively. Solubility study of Ebastine in oil reveals that Rice bran oil shows very good solubility as compared to other oils. Depending on the solubility study of Ebastine in surfactant Labrasol, Tween 40, Tween 60, and Span 20 showed more solubility compared with other surfactants. Hence, these surfactants were selected for further study. Depending on the solubility study of Ebastine in co-surfactants, Transcutol P, Caprol 90 and PEG 600 showed more solubility compared with others.[14-16]

Pseudoternary Phase Diagrams

Pseudo ternary phase diagram was constructed to investigate the effect of surfactant to co-surfactant ratio (km) on the area of microemulsion existence region. It was a well-known fact that Km value has considerable effect on the area of microemulsion existence. The lipid mixture with different surfactant, co-surfactant, and oil ratio leads to the formation of self-emulsifying oil formulations with different properties structure. To form self-emulsifying o/w and w/o microemulsion; oil, a blend of two surfactants, and an aqueous phase were used. These four component systems can be best described by pseudo ternary phase diagram where a constant ratio of two of the components was used and others two were varied. To determine optimum concentration of oil, surfactant, and co-surfactant, for the development of self-emulsifying oil formulations, optimum ratio of excipients concentration established using phase diagram studies provided the area of the monophasic region.[17] A pseudo ternary phase diagram of the investigated system Rice bran oil (oil), Tween 40 (surfactant), Caprol 90 (co-surfactant) are shown in Figure 4.

For the construction of pseudo ternary phase diagram, different ratio of surfactant and co-surfactant was prepared (Smix). Ratios were prepared as follows- oil and Smix (1:1), oil and Smix (2:1), oil and Smix (3:1), and oil and Smix (4:1) as shown in Figures 4-7 respectively.

Water was added in a dropwise manner to each oily mixture under magnetic stirring at 37°C until the mixture became clear at a certain point. Phase diagram indicated that the Smix ratio 3:1 and 4:1 shows less self-emulsification region than the others, therefore, these ratios were rejected. In the case of the phase diagram indicating the Smix ratio 1:1 and 2:1, there is slight difference in the self-emulsification region, but the Smix ratio 1:1 shows larger self-emulsification region than the Smix ratio 2:1. Therefore, the Smix ratio 1:1 will be selected for further study.

Preliminary Screening of Oils, Surfactants

The components were selected for further studies depending on the maximum drug solubility in oil phase and surfactant.

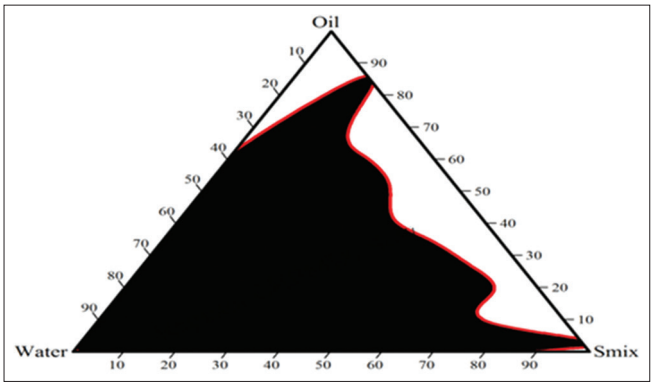

Figure 4: Phase diagram of Rice bran oil, Tween 40, Caprol 90 (1:1) and water

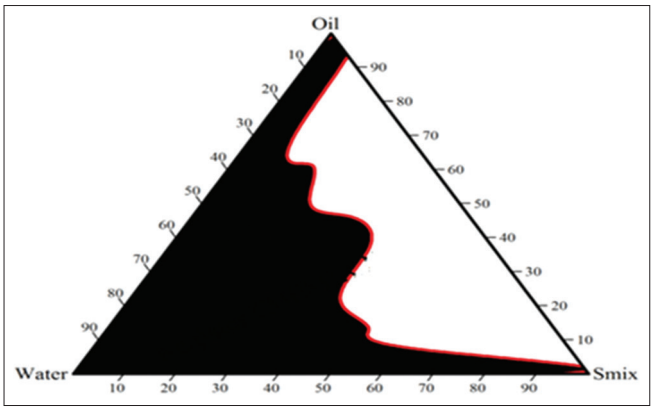

Figure 5: Phase diagram of Rice bran oil, Tween 40, Caprol 90 (2:1) and water

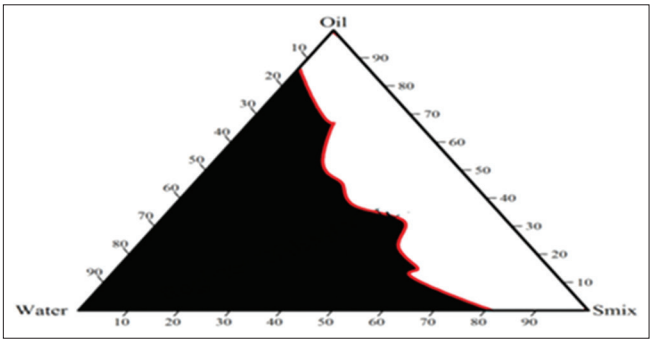

Figure 6: Phase diagram of Rice bran oil, Tween 40, Caprol 90 (3:1) and water

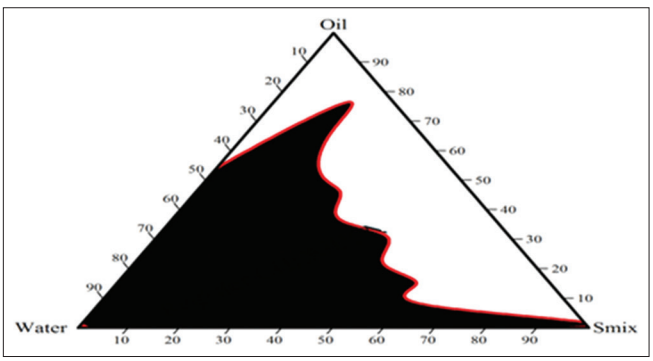

Figure 7: Phase diagram of Rice bran oil, Tween 40, Caprol 90 (4:1) and water

The oil (Rice bran oil) and surfactant (Labrasol, Tween 40, Tween 60, Span 20) having good solubilizing capacity for Ebastine were selected for studying their micro-emulsifying properties. Micro-emulsifying ability of Rice bran oil with different surfactant such as Labrasol, Tween 40, Tween 60, and Span 20 was determined.

Micro emulsion properties of Rice bran oil with Labrasol, Tween 40, and Span 20 was found to be good. These combinations of oil and surfactants show clear phase, i.e., no phase separation is observed. Whereas the combinations of Rice bran oil with Tween 60 show turbidity after 2 h. Hence, this phase was not selected for the further study. The selected phase of oil and surfactant were further used for the preliminary screening of the co-surfactant.[15]

Preliminary Screening of Co-surfactant

According to screening of oil and surfactant three phases were selected as follows:

- Rice bran oil and Labrasol
- Rice bran oil and Tween 40
- Rice bran oil and Span 20.

To these three phases, the co-surfactant that show more solubility with Ebastine were added and microemulsifying properties were studied as shown in Table 1. Combination of Rice bran oil and Labrasol with different co-surfactant was studied depending on the emulsification property. Depending on the preliminary screening of oil, surfactant and co-surfactant suitable final phase were selected for further study as shown in Table 2 and Figure 8.

Preparation of Self-emulsifying Oil Formulations

A series of self-emulsifying oil formulations were prepared using S/CoS combination (Tween 40 and Capryol 90) and Rice bran oil as the oil. In all the formulations, the level of Ebastine was kept constant. Briefly, accurately weighed Ebastine was placed in a glass vial, and oil, surfactant, and cosurfactant were added. Then, the components were mixed by gentle stirring and vortex mixing and were heated at 40°C on a magnetic stirrer, until Ebastine was perfectly dissolved. The mixture was stored at room temperature.[18]

Table 1: Preliminary selected components from solubility stud

Preliminary selected components for self-emulsifying oil formulations		
Oils	**Surfactants**	**Co-surfactants**
Rice bran oil	Labrasol	Transcutol P
Capmul NCM EP	Tween 40	Caprol 90
Captex 300 EP/NF	Tween 60	PEG 600
Cotton seed oil	Span 20	-

Table 2: Finally selected components for the self-emulsifying oil formulations

Ingredients	Quantity in % w/w		
	E1	**E2**	**E3**
Drug (Ebastine)	5	5	5
Rice bran oil	33	34.5	36
Tween 40	31	30.25	29.50
Caprol 90	31	30.25	29.50
% Total	100	100	100

Freeze Thawing

Freeze thawing was performed to evaluate the stability properties of formulations. The formulations were kept for 3–4 freeze-thaw cycles, which involves freezing at 4°C for 24 h and then thawing at 40°C for 24 h. Centrifugation was performed at 3000 rpm for 5 min. The formulations were then observed for phase separation. Formulations which results in stable to phase separation were selected for further studies.

Emulsion Droplet Size Analysis

One hundred microliters of each self-emulsifying oil formulation was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle size analysis (using Malvern Mastersizer, equipped with 2000 Hydro MU) with a particle size measurement range of 0.02–2000 m. All studies were repeated in triplicate, with good agreement being found between measurements.

Self-emulsification and Precipitation Assessment

Different compositions of self-emulsifying oil formulation were categorized on speed of emulsification, clarity, and apparent stability of the resultant emulsion. Visual assessment was performed by dropwise addition of the self-emulsifying oil formulation into 250 mL of distilled water. This was done in a glass beaker at room temperature, and the contents were gently stirred magnetically at ~100 rpm. Precipitation was evaluated using visual inspection of the resultant emulsion after 24 h. The formulations were then categorized as clear (bluish tinge), nonclear (turbid), stable (no precipitation at the end of 24 h), or unstable (precipitation within 24 h).

In Vitro Dissolution Studies

The quantitative *in vitro* release test was performed in 900 mL of buffer pH 1.2 using US Pharmacopeia XXIV

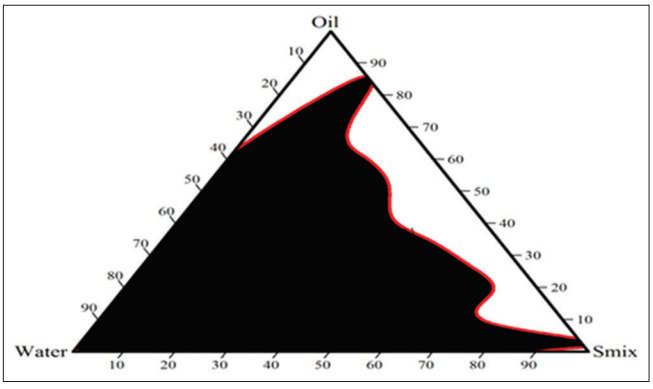

Figure 8: Phase diagram showing liquid self-emulsifying oil formulation

dissolution apparatus Type 2. The paddles were rotated at 100 rpm. The solid self-emulsifying oil formulations were put into hard gelatin capsules (0 sizes) and used for drug release studies; results were compared with those of plain Ebastine. During the release studies, a 5-mL sample of medium was taken out and subjected to drug analysis using ultraviolet (UV) visible spectrophotometer. The removed sample was replaced each time with 5 mL of fresh medium solution. For the determination of the *in vitro* dissolution of plain Ebastine, the medium was changed to buffer pH 1.2 containing Tween 80 (equivalent to the amount used in the formulation). Dissolution studies were also performed in other media (buffer pH 4.5 and 7.2) to examine the effect of pH on drug release.[19]

Stability Studies

The solid self-emulsifying oil formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were kept in stability chambers (Thermolab, Mumbai, India) with humidity and temperature control. They were withdrawn at specified intervals for the analysis over 6 months for intermediate and accelerated conditions and 12 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated by UV spectrophotometric method.

RESULTS AND DISCUSSION

Solubility Studies

To avoid precipitation of the drug on dilution in the gut lumen *in vivo* is important. Components which were used in the system should have high solubilization capacity for the drug, ensuring the solubilization of the drug in dispersion. Results from solubility studies are reported in Figures 1-3. As shown in Figures 1-3, ricebran oil showed the highest solubilization capacity for Ebastine. Thus, for our study, we selected ricebran oil as oils and Labrasol and Transcutol P as surfactant and cosurfactant, respectively.

Pseudoternary Phase Diagrams

Self-emulsifying oil formulation system forms oil-water emulsions with only gentle agitation, on their introduction into aqueous media. Surfactant and cosurfactant are responsible for reducing the interfacial energy as well as providing a mechanical barrier to coalescence. This decrease in the free energy improves the thermodynamic stability of the microemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion.

Drug-excipient Compatibility Study

Visual method

Drug-excipients compatibility study was done for 4 week at 25°C (reaction time), 40°C and samples were visually observed after 2–4 weeks for any color change. The visual observation shows that there was no color change.

Fourier-transform infrared (FT-IR) spectroscopic method

After 4 week, samples were taken to determine any functional group change during the storage. No change in functional peaks of drug observed after 4 week.

Preparation of Liquid SMEDDS (Liquid Self-emulsifying Oil Formulations)

Drug loading

The liquid self-emulsifying oil formulation containing 0.5–5% w/w varying in concentration of drug does not show any precipitation of drug while the liquid self-emulsifying oil formulation containing 5.5% w/w and 6% w/w drug shows precipitation within 12 h. Thus, in liquid self-emulsifying oil formulations, maximum 5% w/w drug can be loaded without any precipitation. Hence, the liquid self-emulsifying oil formulation were formulated using 5% w/w drug and varying concentration of oil, surfactant, and co-surfactant to obtain a formulation showing good self-emulsification efficiency and minimum globule size.

Development of liquid self-emulsifying oil formulations

Drug loading in liquid self-emulsifying oil formulation shows that maximum 5% w/w drug can be loaded in to the self-emulsifying oil formulation. Hence, for further study, different formulations containing 5% w/w drug were selected with the varying concentration of oil, surfactant:co-surfactant (1:1). System with the highest water, absorption capacity was selected for further formulation and also system showing larger microemulsion region. The formulation prepared by selecting composition in the region.

Table 3: Emulsifying properties of oil in combination with different surfactants

Oil	Surfactants	Observation
Rice bran oil	Labrasol	Clear phase
Rice bran oil	Tween 40	Clear phase
Rice bran oil	Tween 60	Turbidity
Rice bran oil	Span 20	Clear phase

Table 4: Emulsifying properties of co-surfactant with selected oil and surfactant

Oil	Surfactant	Co-surfactant	Observation
Rice bran oil	Labrasol	Transcutol P	E
		Caprol 90	E
		PEG 600	NA
Rice bran oil	Tween 40	Transcutol P	E
		Caprol 90	ME[a]
		PEG 600	E
Rice bran oil	Span 20	Transcutol P	ME[b]
		Caprol 90	ME[b]
		PEG 600	E

ME[a]: Formulation spreads rapidly in water-forming clear and transparent micro emulsion in<1 min, ME[b]: Formulation formed transparent, gel-like intermediate structure prior to dispersing completely but could form microemulsion in 3–5 min, E: Formulation droplet spreads in water to form turbid emulsion in>5 min, NA: Formulation droplet spreads in water to form turbid emulsion

Characterization of Liquid Self-emulsifying Oil Formulations

Emulsification efficiency

Emulsification property of liquid self-emulsifying oil formulation was determined by diluting self-emulsifying oil formulation 100 times with distilled water, continuously stirring on magnetic stirrer.

Liquid self-emulsifying oil formulation E1 spreads rapidly, form transparent gel-like intermediate structure before form emulsion. Liquid self-emulsifying oil formulation E2 spreads rapidly in water forming clear and transparent emulsion. Liquid self-emulsifying oil formulation E2 spreads rapidly in water forming transparent emulsion with white ting-like structure.

The study of emulsifying property of different liquid formulation [Tables 3 and 4] reveals that all three formulation shows good emulsifying property, E3 formulation forms whitish emulsion so, it may be rejected.

Precipitation assessment

The formulated self-emulsifying oil formulation diluted with the 100 ml of distilled water and the diluted self-emulsifying oil formulation observed for the precipitation.

From precipitation assessment, E1 and E2 formulations found to be transparent, clear emulsion with no precipitation and stable. E3 formulation forms whitish precipitate after 12 h. Therefore, E3 formulation rejected. E1 and E2 formulation were selected for the study as shown in Table 5.

Drug content determination

Amount of drug present in the liquid self-emulsifying oil formulation was determined using UV Spectroscopic method as shown in Table 6.

Self-emulsification time

The results obtained for self-emulsification time were noted. The E2 formulation required less time for emulsification than E1 formulation. Both formulations have good tendency for self emulsification.

Table 5: Precipitation assessment of different liquid self-emulsifying oil formulations

Formulation code	Precipitation after 24 h
E1	Transparent, clear emulsion, no precipitation, stable
E2	Transparent, clear emulsion, no precipitation, stable
E3	Precipitation observed after 24 h

Table 6: Drug content of different liquid self-emulsifying oil formulations

Formulation code	Drug content (% w/w)
E1	92.87±0.36
E2	96.02±0.64
E3	95.41±0.85

Table 7: *In vitro* drug release study of Ebastine and liquid self-emulsifying oil formulations

Time (min)	% Drug release		
	Plain drug	E1	E2
5	0.74±0.014	44.960±0.747	45.20±0.601
10	3.20±0.055	77.756±0.211	79.32±0.177
20	6.26±0.134	91.644±0.109	94.25±0.232
30	8.58±0.172	94.962±0.905	95.63±0.205
40	12.21±0.312	95.723±0.273	95.97±0.113
50	15.67±0.375	96.169±0.232	99.18±0.752
60	23.12±0.685	97.38±0.352	99.79±0.168

Table 8: Globule size of liquid self-emulsifying oil formulations

Formulation code	Globule size	Polydispersity index
Liquid self-emulsifying oil formulation (E2)	286.2 nm	0.857

Conclusion

From all above characterization, it was found that E2 formulation is better than E1 formulation. Therefore, E2 formulation considered to be the final formulation for the study.

Drug Release Study

In SMEDDS, the free energy required to form an emulsion is very low, thereby allowing spontaneous formation of an interface between the oil droplets and water. It was suggested that the oil/surfactant/co-surfactant and water phase effectively decreases the oil droplet size and eventually increases the release rate. *In vitro* drug release study was performed for Ebastine self-emulsifying oil formulation and plain drug as shown in Figure 9 and Table 7.

Table 9: Zeta potential measurement of liquid self-microemulsifying drug delivery systems

Formulation	Zeta potential	Electrophoretic mobility
Liquid self-emulsifying oil formulation (E2)	2.6 mV	0.000020 cm^2

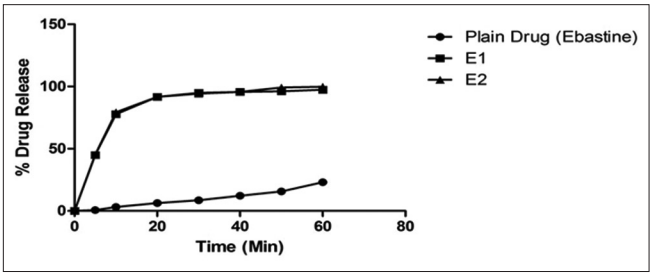

Figure 9: Comparison of dissolution profile of liquid self-emulsifying oil formulation (E1 and E2) and plain drug Ebastine

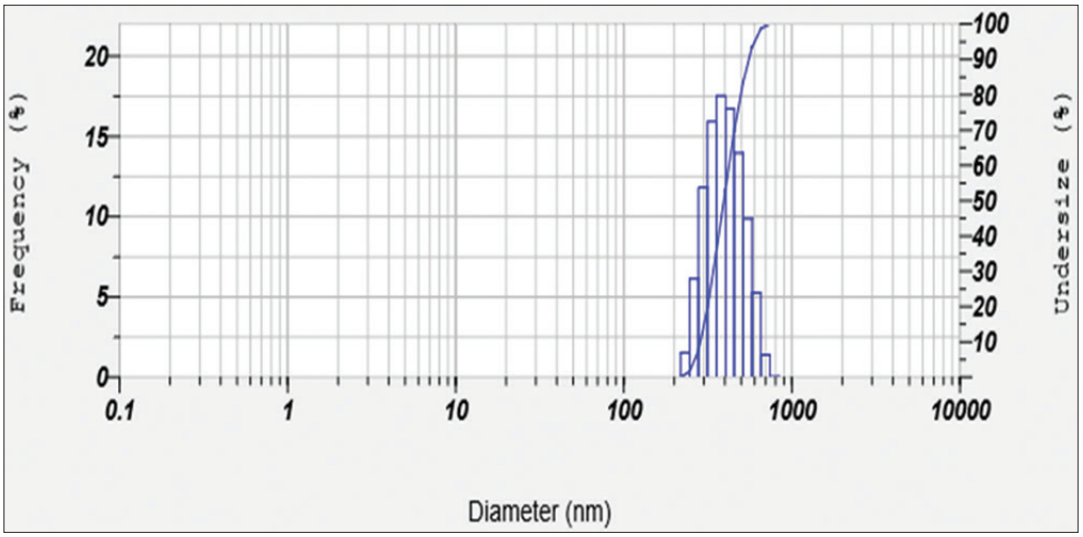

Figure 10: Histogram of globule size of liquid self-emulsifying oil formulation

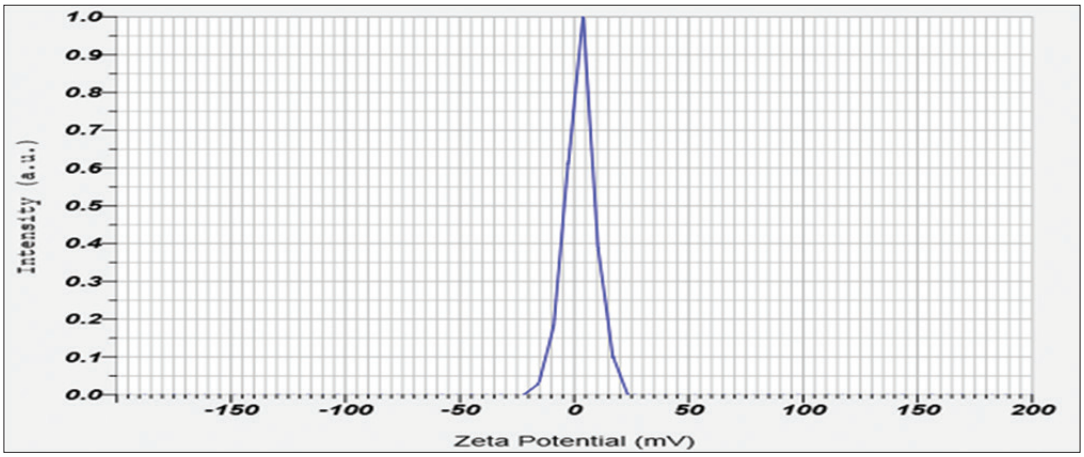

Figure 11: Zeta potential measurement of liquid self-emulsifying oil formulation

In vitro release study, results reveals that only 23.12% w/w drug was released from plain Ebastine filled in capsule in 60 min, whereas 97.38% w/w, 98.79% w/w drug release from the liquid self-emulsifying oil formulation (E1, E2), respectively, within 60 min.

Measurement of Mean Globule Size

The droplet size of the emulsion determines the rate and extent of drug release as well as absorption. The globule size of self-emulsifying oil formulation is shown in Table 8 and Figure 10.

Zeta Potential Measurement

The zeta potential was used to identify the charge on the droplet. The values of zeta potential indicate the degree of electrostatic repulsion between particles in the dispersion as shown in Figure 11 and Table 9.

Preparation of Solid SMEDDS

As E2 self-emulsifying oil formulation passes all the evaluation parameters and also shows very good emulsifying property, it was selected as final formulation and it was converted into solid self-emulsifying oil formulation. Liquid self-emulsifying

Figure 12: (a and b) Preparation of solid self-emulsifying oil formulation by spray drier

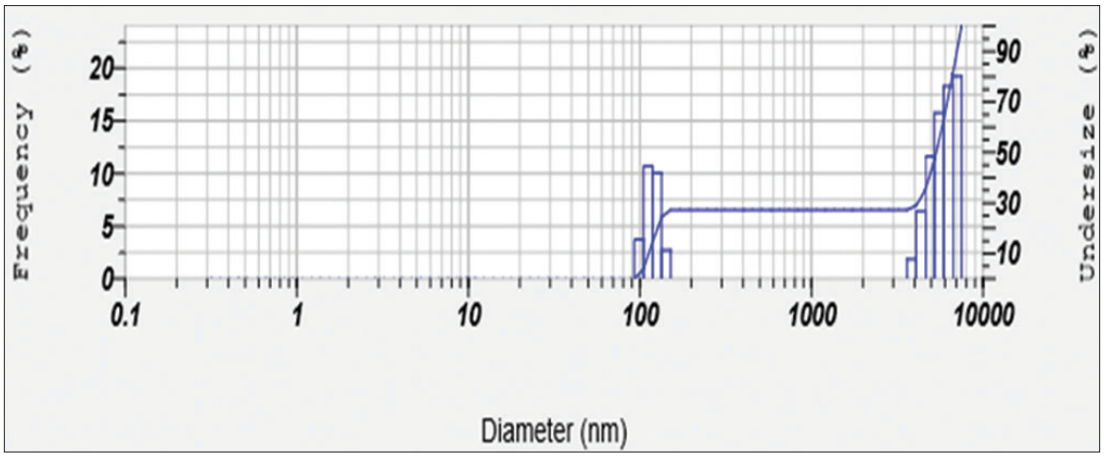

Figure 13: Histogram of particle size distribution of solid self-emulsifying oil formulation

Table 10: Particle size and polydispersity index of solid-self-micro-emulsifying drug delivery systems

Formulation	Particle size (nm)	Polydispersity index
Solid self-emulsifying oil formulation	260	0.571

Table 11: Flow properties of spray dried self-emulsifying oil formulation

Parameter	Result	Inference
Bulk density	0.769±0.0112 g/ml	-
Tapped density	1.000±0.0123 g/ml	-
Carr's index	30.03	Poor
Hausner ratio	1.300	Passable
Angle of repose	34.88	Passable

oil formulation, E2 formulation was converted in to solid self-emulsifying oil formulation by spray drying technique [Figure 12] using Aerosil 200 (colloidal silicone dioxide) as a carrier. A total of 10 g of liquid self-emulsifying oil formulation contains 500 mg of drug Ebastine. After addition of 5 g of Aerosil 200 as carrier for spray drying, the total weight 15 g contains 500 mg of drug Ebastine. Therefore, by calculation 300 mg of spray-dried powder contains 10 mg of Ebastine as a dose of drug.

Reconstitution Properties of Solid Self-emulsifying Oil Formulation

Visual observation

Reconstitution property of solid self-emulsifying oil formulation was determined by stirring solid self-emulsifying oil formulation with distilled water for 5 min and observed visually. Solid self-emulsifying oil formulation showed rapid dispersion without any lump or agglomeration. This dispersion when observed visually after incubation for 60 min at room temperature was well dispersed without phase separation.

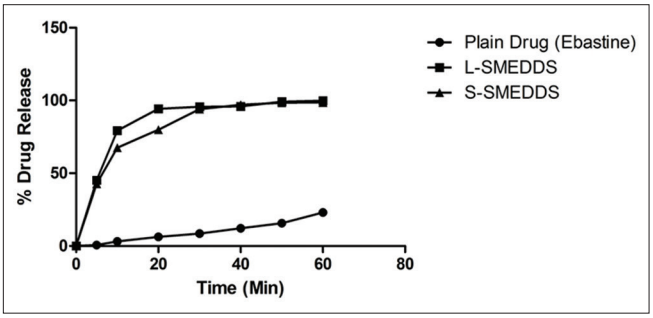

Figure 14: Comparison of dissolution profile of plain drug, liquid self-emulsifying oil formulation and solid self-emulsifying oil formulation

Particle size determination

Particle size and polydispersity index of solid self-emulsifying oil formulation was observed as shown in Figure 13 and Table 10 respectively.

Characterization of Solid Self-emulsifying Oil Formulation

Yield of spray-dried product

The percentage yield of solid self-emulsifying oil formulation was calculated by using following formula:

$$\% \text{ Yield } = \frac{\text{Material recovered from spray dryer}}{\text{Weight of liquid SEOF} + \text{Weight of Aerosil 200}} \times 100$$

$$= \frac{13.95}{10+5} \times 100 = 93.00\%$$

The percentage yield of spray-dried solid self-emulsifying oil formulation product was found to be 93.00% w/w. The solid self-emulsifying oil formulation obtained with set parameter such as inlet, outlet temperature, and feed rate was satisfactory considering the small batch.

Powder flow properties

Spray-dried product was evaluated for bulk density, tapped density, Carr's compressibility index, Hausner ratio, angle of repose.

Table 12: *In vitro* dissolution data of plain drug, liquid self-emulsifying oil formulation, solid self-emulsifying oil formulation

Time (min)	% Drug release		
	Plain drug	Liquid self-emulsifying oil formulation	Solid self-emulsifying oil formulation
0	0	0	0
5	0.74±0.014	45.20±1.395	42.76±1.141
10	3.20±0.055	79.32±1.177	67.57±1.202
20	6.26±0.134	94.25±1.234	79.99±1.385
30	8.58±0.172	95.63±1.208	94.08±1.237
40	12.21±0.312	95.97±1.113	97.07±1.077
50	15.67±0.375	99.18±1.752	98.45±1.098
60	23.12±0.685	99.79±1.168	98.66±1.388

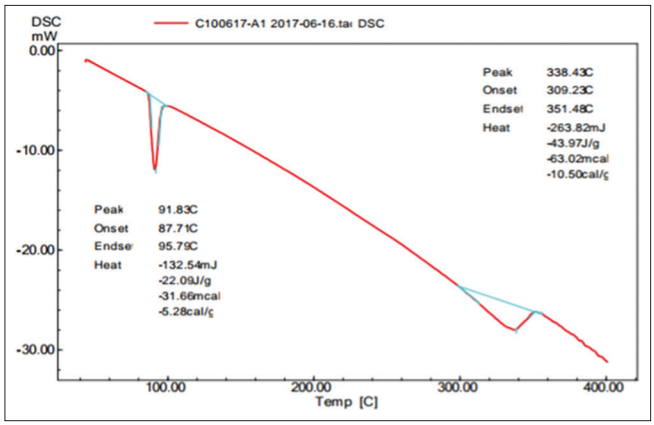

Figure 15: Differential scanning calorimetry of Ebastine

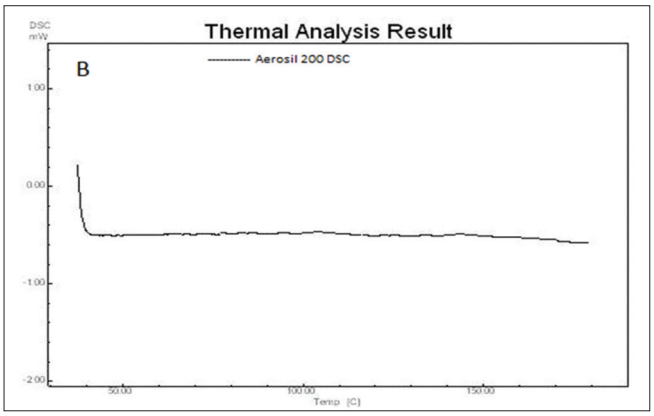

Figure 16: Differential scanning calorimetry of Aerosil-200

The flow property of solid self-emulsifying oil formulation was found to be passable as shown in Table 11 because of fluppy mass mass of Aerosil 200 and also it contain oil, surfactant, and co-surfactant adsorbed on Aerosil 200.

Drug Content Determination

In vitro dissolution study

In vitro dissolution profile of plain drug, liquid self-emulsifying oil formulation, and solid self-emulsifying oil formulation were compared together together as shown in Figure 14 and Table 12. *In vitro* study was performed in three batches with mean and standard deviation. Liquid self-emulsifying oil formulation shows more percentage drug release than the solid self-emulsifying oil formulation and plain drug.

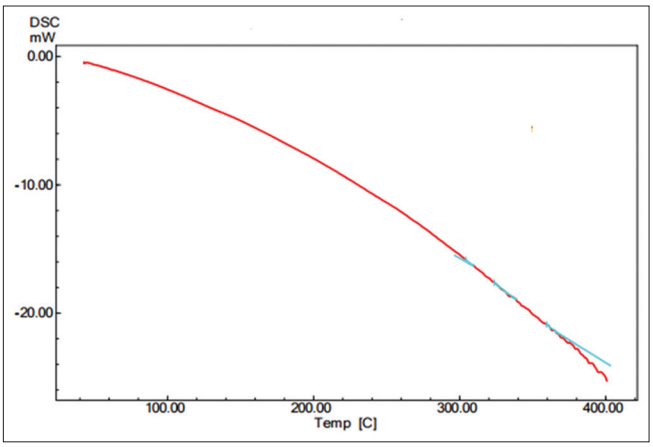

Figure 17: Differential scanning calorimetry of solid self-emulsifying oil formulation

Solid State Characterization of Solid Self-emulsifying Oil Formulation Powder

Differential scanning calorimetry (DSC)

DSC of Ebastine, Aerosil 200 and solid self-emulsifying oil formulation were performed as shown in Figures 15-17. DSC of Ebastine exhibits a sharp melting point at 91.83°C with onset at 87.71°C and end set or recovery at 95.79°C. The DSC of solid self-emulsifying oil formulation does not show the sharp peak. The absence of sharp melting peak of Ebastine in the range 87–91°C of solid self-emulsifying oil formulation indicates that the lipids and Aerosil 200 inhibits the crystallization of Ebastine, i.e., Ebastine is in amorphous form or in solubilized form in solid self-emulsifying oil formulation.

Scanning electron microscopy (SEM)

SEM was used to determine the particle morphology of pure drug (Ebastine) and optimized self-emulsifying oil formulation. The SEM of Ebastine, Aerosil 200 and solid self-emulsifying oil formulation were done as shown in Figures 18-20.

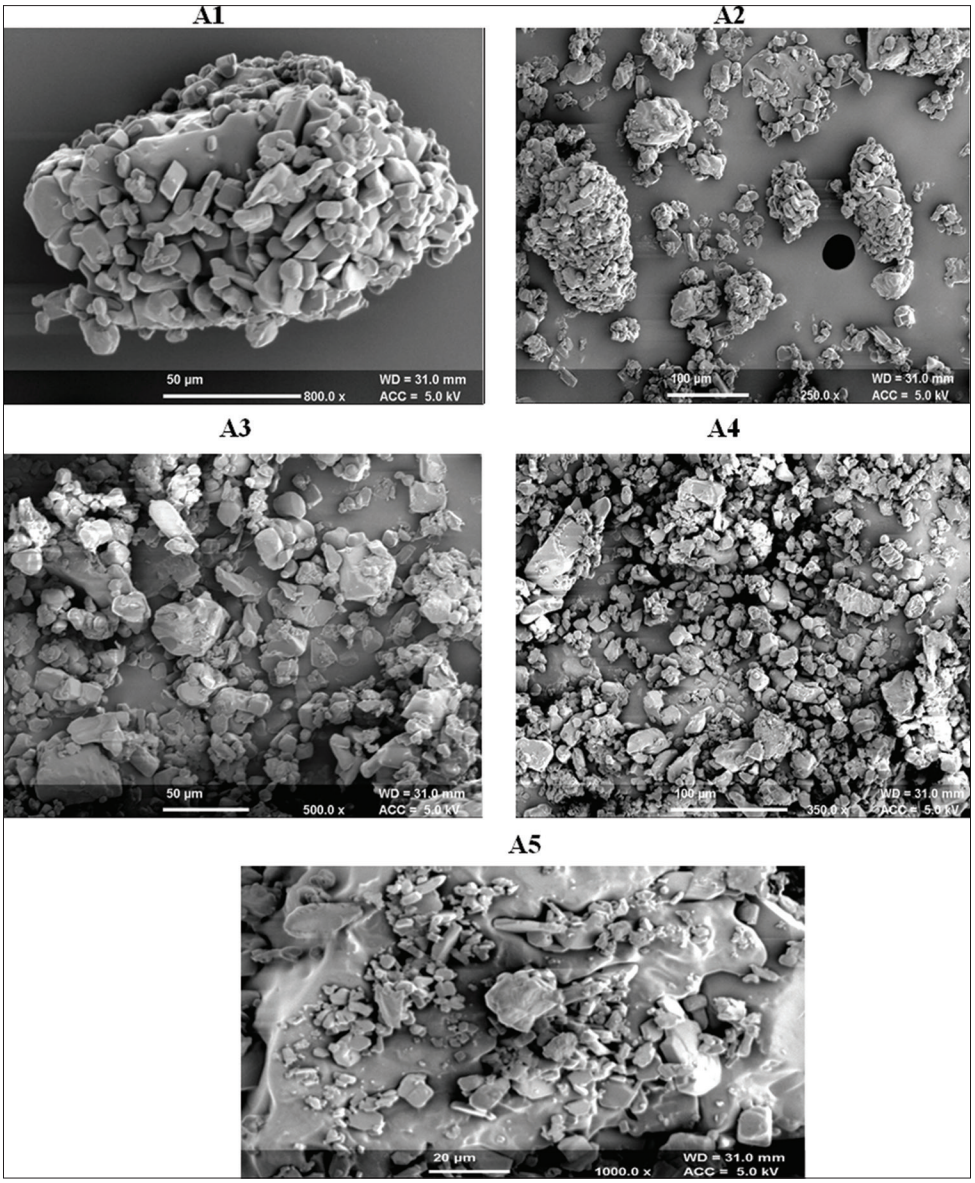

Figure 18: Scanning electron microscopy of Ebastine (A)

Results reveal that the Ebastine present as rough surface and irregular shape particles. Aerosil 200 (Colloidal silicon dioxide) was detected as smooth surface sphericle particles. SEM of the solid self emulsifying oil formulation does not show any irregular shape particles of drug (Ebastine) on the surface of Aerosil 200 indicate that drug was present in the soluble form in lipid (self emulsifying oil formulation) , which was absorbed on the surface of Aerosil 200.

Powder X-ray diffraction (XRD)

The absence of sharp peak had been observed in Aerosil 200 and solid self-emulsifying oil formulation. The absence of sharp peak as seen in Figures 21 and 22 in solid self-emulsifying oil formulation reveals that the drug (Ebastine) is present in the amorphous form or present in solubilized form in solid self-emulsifying oil formulation XRD of Ebastine was performed as seen in Figure 23.

Stability Study of Liquid Self-emulsifying Oil Formulation

Thermodynamic stability studies

Thermodynamic study reveals that there was no change in the formulation during the stability study for 3 months.

Centrifugation test

Self-emulsifying oil formulation was centrifuged at 3500 rpm for 30 min using centrifuge (Remi motors Ltd.), no phase separation found. This proves that the liquid self-emulsifying oil formulation were stable when subjected to centrifugation test.

Stability study of solid self-emulsifying oil formulation

Stability study of solid self-emulsifying oil formulation at Freeze temperature (−20°C), room temperature (25°C), and high temperature (40°C) was done [Figure 24] for 3 months and evaluated for the following parameters.

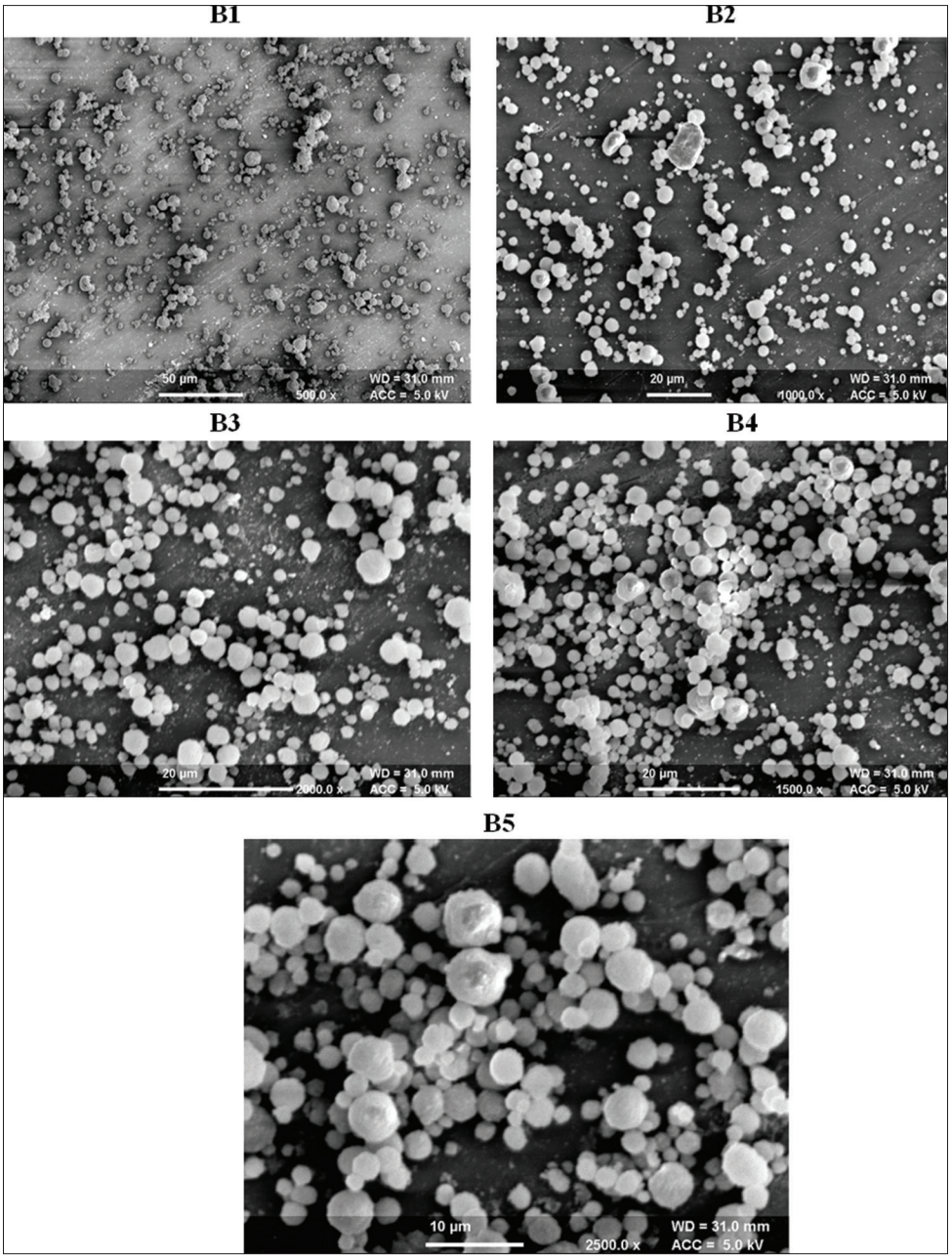

Figure 19: Scanning electron microscopy of Aerosil 200 (B)

Visual observation

Visual observation study reveals that there was no change in color observed during stability study for 3 months.

Drug content

Drug content of solid self-emulsifying oil formulation was done after each month for 3 months and results are shown in Table 13. The drug content data show that there was no change in drug content of solid self-emulsifying oil formulation. This proves that the solid self-emulsifying oil formulations were stable.

Emulsification property

Emulsifying property of solid self-emulsifying oil formulation was done after each month for 3 months and results are shown in table. There was no change in the emulsifying property; this shows that the solid self-emulsifying oil formulations were stable.

In vitro drug release study

In vitro drug release study of solid self-emulsifying oil formulation was done after 3 months in 0.1N HCl solution.

In vitro drug release study does not show any major change. This proved that solid self-emulsifying oil formulations were stable.

FT-IR study

FT-IR of solid self-emulsifying oil formulation sample placed at different temperature (−20°C, 25°C, 40°C) was done

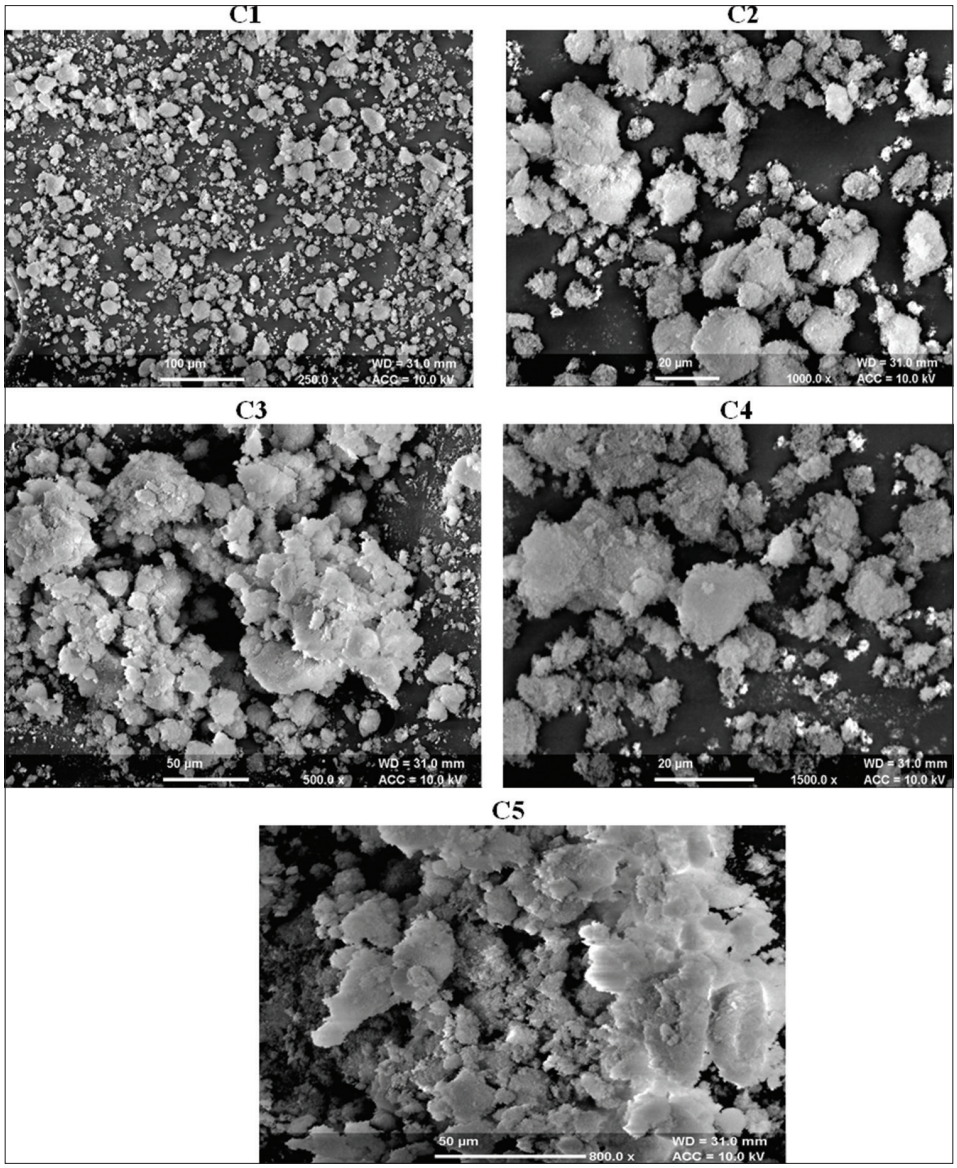

Figure 20: Scanning electron microscopy of solid self-emulsifying oil formulation (C)

Table 13: Drug content in solid self-emulsifying oil formulation

Formulation	% Drug content
Solid self-emulsifying oil formulation	95.41±2.485

after 3 months to observe any change in drug. The FT-IR of Solid self-emulsifying oil formulation shows that there was no change in the functional peaks of drug (Ebastine) at all temperature after 3 months.

Antiallergic/antihistaminic activity

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa. It induces an immunoglobulin e-mediated reaction resulting from inflammation of the airway mucosa with hypersensitivity caused by seasonal or perennial responses to specific allergens. Approximately 500 million people are affected by AR worldwide, and it presents with symptoms of sneezing, itching, and respiratory obstruction causing pain. Besides these symptoms, AR can also lead to other inflammatory diseases such as asthma, rhino sinusitis, allergic conjunctivitis, otitis media with effusion, nasal polyp, tubal dysfunction, and adenoid hypertrophy.

Elicitation and group classification of AR model

The 6-week-old female mice were divided into five groups (n = 6 per group): (1) Control group; (2) ovalbumin (OVA) sensitized (OVA) group; (3) OVA + Ebastine (plain drug) group; (4) OVA + Ebastine formulation group; and (5) OVA + cetirizine-treated (Cet) group. Mice were then stabilized for 1 week; they were OVA-sensitized by intraperitoneal injection of 50 mg OVA (chicken egg albumin) in 200 mL of phosphate-buffered saline containing 2 mg aluminum hydroxide on day 0, day 7, and day 14. 1 week after the last injection on day 21, the mice were given with 20 mL phosphate-buffered saline containing 50 mg/mL OVA into the nasal cavities. From day 21 to day 31, mice in the control and OVA groups were given saline by peroral administration, and those in the Cet group received 10 mg/kg,

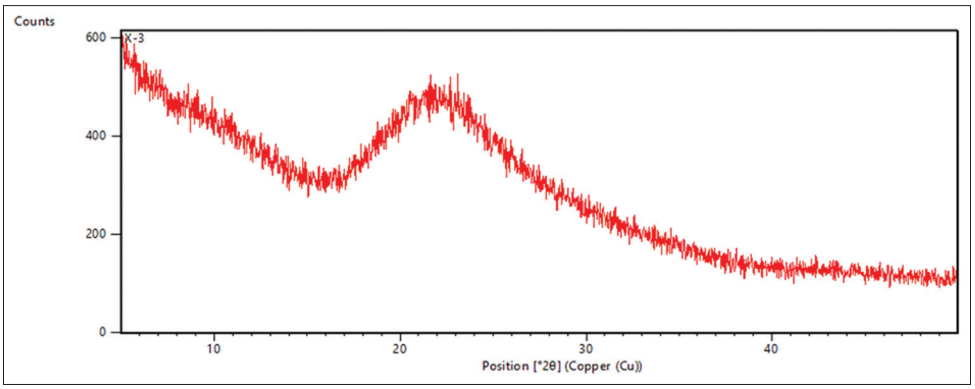

Figure 21: X-ray diffraction of Aerosil 200

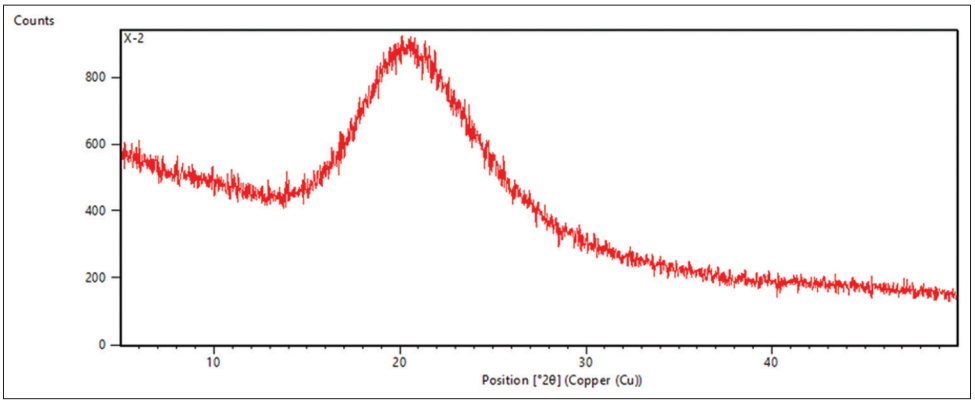

Figure 22: X-ray diffraction of solid self-emulsifying oil formulation

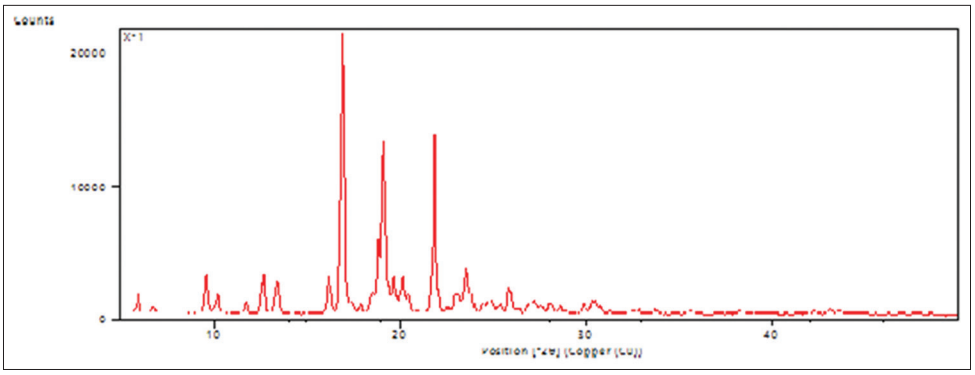

Figure 23: X-ray diffraction of Ebastine

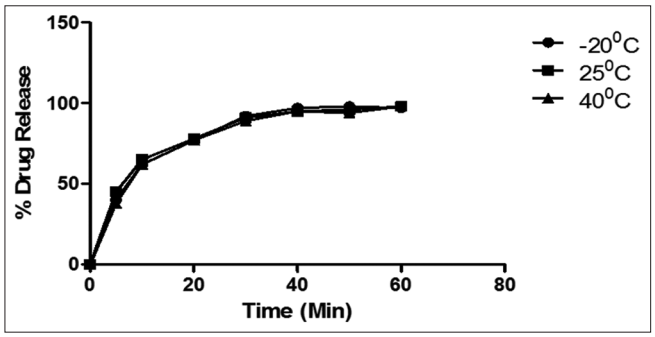

Figure 24: *In vitro* dissolution data of solid self-emulsifying oil formulation after 3 months

cetirizine hydrochloride, and the Ebastine formulation group was orally administered 10 mg/kg Ebastine formulation 1 h before intranasal challenge of OVA under the same conditions. In this study, cetirizine was used as a positive control.

Nasal symptom evaluation

The mice (6 weeks old, female) was administrated OVA intranasally, and the nasal symptoms was evaluated 2 min later by counting the time of nasal rubbing and number of sneezing events for 10 min. This procedure was carried out for 10 days starting from day 21. 3 h after the last observation, the mice were euthanized with sodium pentobarbital results shown in Figure 25.

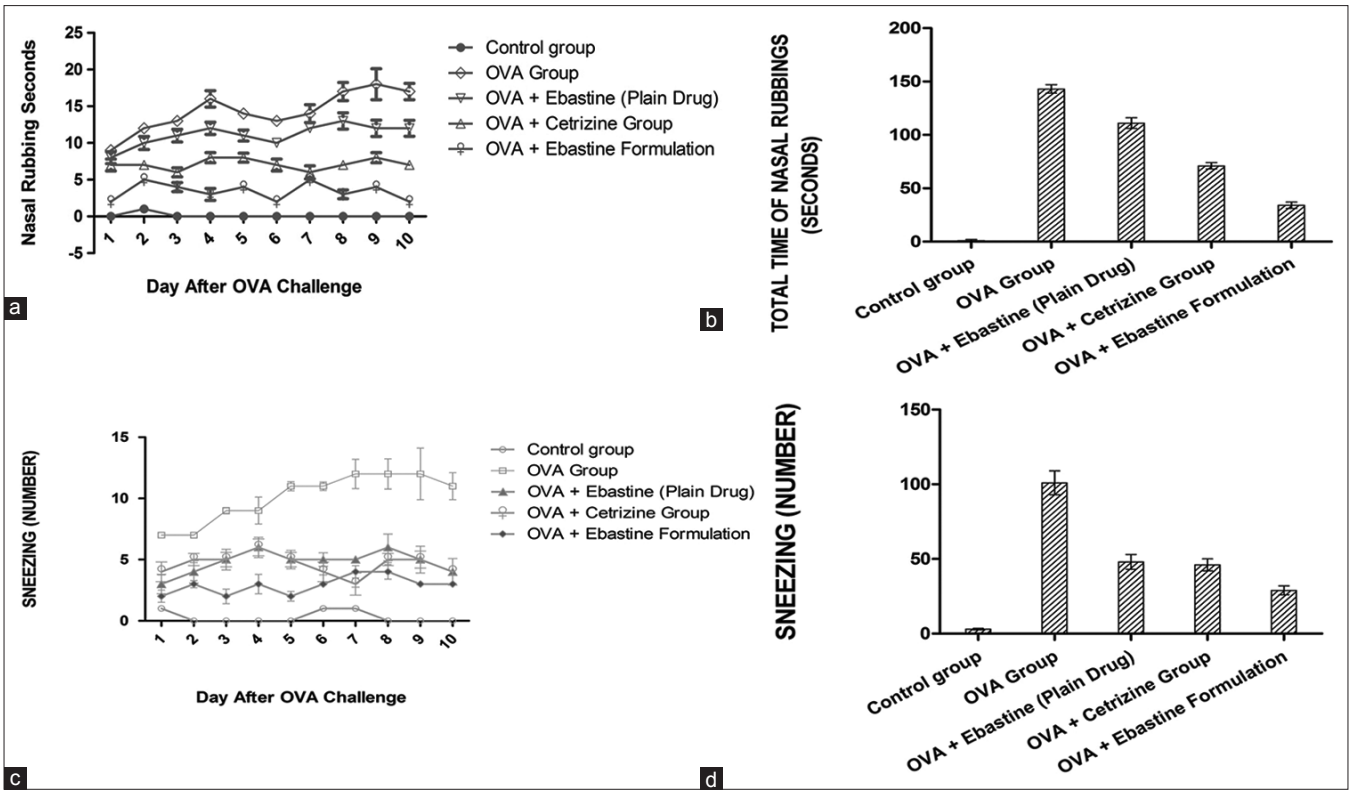

Figure 25: Time course of the development of nasal rubbing and sneezing induced by antigen in mice sensitized with ovalbumin and aluminum hydroxide. Clinical scores such as sneezes and nasal rubs were measured for 10 min after the last intranasal challenge from day 21 to day 30. (a) Time of nasal rubbing each day. (b) Total time of nasal rubbing for 10 days. (c) Number of sneezes each day. (d) Total number of sneezes for 10 days. Data represent the mean ± standard error

DISCUSSION

In the present investigation, an attempt has been made to formulate and evaluate solid SMEDDS (solid self-emulsifying oil formulation) containing oil, surfactant, and co-surfactant. Ebastine is practically insoluble in water, gastric fluid, and having low oral bioavailability due to slow dissolution rate. The bioavailability problem can be overcome by formulating SMEDDS. Self-emulsifying oil formulations are isotropic mixture of oil, surfactant, and co-surfactant which forms o/w micro emulsion on dispersion in to the aqueous phase by gentle agitation of the gut.[20]

In the present study, SMEDDS of Ebastine was formulated by using combination of oil, surfactant, and co-surfactant. On aqueous dilution of this system, spontaneously emulsification forms an o/w micro emulsion. This property depends on the composition of the excipients as well as their individual concentration in the mixture. The selection and screening potential components was done on the basis of the solubility of the drug in the excipients (oil, surfactant, and co-surfactant) and emulsifying properties of those excipients in combination. Different excipients were evaluated for the solubility of the drug and emulsifying property. A particular self-microemulsifying mixture comprises Rice bran oil (oil), Tween 40 (surfactant), and Caprol 90 (co-surfactant). Surfactant and co-surfactant were employed in the ratio of 1:1. Phase diagram was constructed to investigate effect of surfactant to co-surfactant ratio on the area of micro emulsion existence region. In the prepared phase drug loading was done liquid self-emulsifying oil formulation were prepared. This prepared liquid self-emulsifying oil formulation were evaluated for emulsification efficiency, precipitation assessment, drug content, drug release, self-emulsification time, refractive index, globule size, and zeta potential. This liquid self-emulsifying oil formulation was converted into solid self-emulsifying oil formulation by spray drying. It was calculated that 300 mg of spray-dried powder content 10 mg of the active drug. The solid self-emulsifying oil formulation were evaluated for the parameters such as powder flow properties, reconstitution properties, particle size, FT-IR, DSC, SEM, XRD, drug content, emulsification properties, drug release, and stability study.

CONCLUSION

Solubility study showed that Rice bran oil, Tween 40, Caprol 90 shows good solubilizing property for Ebastine. The homogeneous mixture of Rice bran oil as oil, Tween 40 as surfactant shows good emulsifying property with Caprol 90 as co-surfactant in 1:1 ratio. The ternary phase diagram of the surfactant:co-surfactant (1:1) ratio shows larger micro emulsifying region than 2:1, 3:1, and 4:1 ratio. The stability of the self-emulsifying oil formulation was improved by spray drying technique. Aerosil 200 was used as adsorbent for converting liquid self-emulsifying oil formulation into solid self-emulsifying oil formulation. By performing the stability study, it was concluded that liquid self-emulsifying oil formulation and solid self-emulsifying oil formulation were stable.

The present study aimed to evaluate the effect of Ebastine solid self-emulsifying oil formulation on OVA sensitization/challenge-induced AR in mice through regulation of the allergic inflammatory response. Nasal symptoms were evaluated in an OVA-induced AR mouse model.

In conclusion, Ebastine self-emulsifying oil formulation is an effective formulation for the treatment of AR.

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