ORIGINAL ARTICLE

Formulation and Evaluation of Self-emulsifying Drug Delivery System of Bosentan

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Abstract

Aim: The objective of the present study was to develop self-emulsifying drug delivery system (SEDDS) of bosentan to improve its solubility, *in vitro* dissolution efficiencies, and further the bioavailability. **Materials and Methods:** The solubility of bosentan in various oils, surfactants, and cosurfactants was determined. Pseudoternary phase diagrams were constructed using Gelucire 44/14, Cremophor EL, and polyethylene glycol 400 (PEG 400) to identify the efficient self-microemulsification region. Prepared SEDDS was evaluated for emulsification time, drug content, optical clarity, droplet size, zeta potential, and *in vitro* dissolution. **Results and Discussion:** The optimized formulation FF5 had shown the smallest particle size, maximum solubility, less emulsification time, good optical clarity drug stability in water, and improved *in vitro* release. In the present study, already existed historical data were used for importing data. In the present study already existed historical data was used for importing data. Optimized SEDDS bosentan oral formulation (FF5) prepared had shown improved *in vitro* release when compared to commercial formulation. **Conclusion:** It was concluded that SEDDS would be a promising drug delivery system for poorly water-soluble drugs through oral route.

Key words: Bosentan, self-emulsifying drug delivery system, Gelucire 44/14, Cremophor EL, pseudoternary phase

INTRODUCTION

Approximately 40% of new drug candidates have poor water solubility, and the oral deliveries of such drugs are frequently associated with low bioavailability.[1] To overcome these problems, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, and micronization. Majority of these approaches have their limitations because of the need for specialized equipment, complicated manufacturing process, longer processing time, and regulatory complexity. Lipid-based formulation approaches, particularly the self-emulsifying drug delivery system (SEDDS), are well known for their potential as an alternative approach for delivery of hydrophobic drugs,[2] which are associated with poor water solubility and low oral bioavailability.[3-5] SEDDS is among the methods used to improve the oral bioavailability of poorly soluble drugs by presenting and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract (GIT).[6]

SEDDSs are the isotropic mixtures of oil, surfactant, cosurfactant, and drug which form oil in water microemulsion.[1] These formulations spread readily in the GIT, and the digestive motility of the stomach and intestine provides the agitation necessary for self-emulsification. In a good self-emulsifying system, small emulsion droplets containing dissolved drug are formed on contact with the gastrointestinal fluid. The drug in the fine emulsion droplets

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is exposed to a large interfacial area, thus allowing for greater diffusion through the membrane to take place.[7]

SEDDS was defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively one or more hydrophilic solvents and cosolvents/ surfactants. On mild agitation followed by dilution in aqueous media such as GI fluids, these systems can form fine oil-in water emulsions or microemulsions.[8-10] It is thought that the microemulsion is spontaneously formed by the combined action of the specific pharmaceutical excipients.[11] The microemulsion droplets dispersed in the GIT provide large surface area and promote a rapid release of the dissolved form of the drug substance and/or mixed micelles containing drug substance, and they may be also responsible for transporting the drug through the unstirred water layer to the GI membrane for absorption. In addition to the enhanced dissolution of drugs by SEDDS, another factor contributing to the increasing bioavailability is that the lymphatic transport is responsible for a portion of the drug uptake.[12] The lipid composition of SEDDS may be related to facilitate the extent of lymphatic drug transport by stimulating lipoprotein formation and intestinal lymphatic liquid flux.[13,14] Since these are SEDDSs, they form emulsion after oral ingestion, and hence, they are less prone to stability issues which indicate that they are more stable than conventional emulsions.

A factorial design[15-17] is an alternative to overcome the unreliable results and improper conclusions besides wastage of production cost and workforce. Based on the principal of the design of experiments, the methodology encompasses the use of various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum formulation(s). The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms. In the present study, historical data were used for importing data that already exist. A design expert version of 10.0.5.0 was used for the present study. The study type was a mixture with historical data as design type, randomized as subtype, and design model was linear. A total of five runs with no blocks were used for the study. Three factors such as Gelucire 44/14, Cremophor EL and polyethylene glycol 400 (PEG 400) were taken and coded as A, B, and C. Optical clarity (%) and cloud points (°C) were taken as responses. By using this, the significance of individual responses and desirability were thus resoluted.

Bosentan chemically is 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy) 2- (pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide. Bosentan is an endothelial receptor antagonist. Bosentan decreases pulmonary vascular resistance. Bosentan is poorly soluble in water and has 45% bioavailability.[18] The low aqueous solubility and poor dissolution of the molecule in gastric fluid affects its rate of absorption, resulting in a low and variable oral bioavailability. The aim of the present study was to develop self-emulsifying delivery system of bosentan to enhance its solubility and dissolution rate, thereby enhancing the bioavailability.

MATERIALS AND METHODS

Materials

Bosentan was a gift sample from Cipla, Mumbai. Gelucire 44/14 and Cremophor EL were procured from Mylan Laboratories, Hyderabad. PEG 400, Tween 80, and Tween 20 were procured from commercial sources. All other materials used were of pharmacopeial grade.

Estimation of bosentan

Bosentan content of the SEDDS was estimated by ultraviolet (UV) spectrophotometric method at 269 nm in water. The method was validated for linearity, precision, and accuracy. The method obeyed Beer's law in the concentration range of 0–12 µg/ml. When a standard drug solution was assayed repeatedly (n = 6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6 and 0.8%, respectively.

Determination of solubility

The solubility of bosentan in various solvents (oils, surfactants, buffers, and cosurfactants) was determined by shake-flask method and sonication. An accurately measured 5 ml of each solvent separately was taken in a vial. To each vial, an excess amount of bosentan was added and properly sealed, stirred using cyclomixer (Remi Laboratory Instruments) for 10 min and then sonicated for a period of 12 h. The solution was equilibrated for 48 h and then centrifuged for 10 min at 2000 rpm using a centrifuge (Eppendorf centrifuge 5415 R). The supernatant was filtered, and subsequent dilutions were made with methanol: chloroform (80: 20 v/v) and analyzed using UV-visible spectrophotometer (M/s Labindia, Mumbai, India) at a wavelength of 269 nm. Blank was prepared by dissolving respective vehicles in methanol: chloroform (80: 20 v/v) and the dilutions were made similar to that of drug samples.

Selection of surfactant and cosurfactant[2]

Screening of surfactant for emulsifying ability was done by % transmission studies. In these studies, 300 w/w of surfactant was added to 300 w/w of selected oily phase (quantities were taken as weights). The mixture was gently heated[19] at 45–60°C for homogenizing the components and were brought to 25- 30oC by keeping at room temperature. The isotropic mixture of 50 mg was accurately weighed and diluted to 50 ml with distilled water to yield a fine emulsion. The emulsions

were then allowed to stand for 2 h, and transmittances were assessed at 300 nm using UV spectrophotometer with distilled water as blank. The emulsion was further observed visually for any turbidity or phase separation. In case of cosurfactant selection, the mixtures of the cosurfactant, selected surfactant, and the selected oil were prepared and evaluated in similar fashion as that of surfactants.

Drug-excipient compatibility studies

Fourier-transform infrared (FTIR) studies

FTIR studies were carried out by scanning (Bruker Vertex 70 spectrometer) the sample in potassium bromide disks. The samples of pure drug and optimized self-emulsifying formulation were scanned individually to investigate the possible interactions.

Visual assessment studies

Bosentan alone and in combination with Gelucire 44/14, Cremophor EL, and PEG 400 and mixture of bosentan, Gelucire 44/14, Cremophor EL, and PEG 400 were observed for visual assessment studies. In these studies the initial and final appearance of the formulation after 4 weeks were recorded by keeping the sample at refrigerator (2-4ºC), room temperature and 40ºC.

Construction of Pseudoternary Phase Diagram[10-19]

Pseudoternary phase diagrams were constructed using aqueous titration method to examine the formation of oil in water emulsions. Based on the solubility study, the oil, surfactant, and co surfactant were selected. Surfactant and cosurfactant (Smix) in each group were mixed in different weight ratios (1:0, 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, and 4:1). These Smix ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for a comprehensive study of the phase diagrams. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from 1:1 to 2:1 in different glass vials. Combinations of oil and Smix 1:1, 1:2, and 2:1 were made for the study and given in the Table 3.

From the data, a series of self-emulsifying formulations were prepared with varying concentrations of oil, surfactant, and cosurfactant. The concentration of Gelucire 44/14 was varied from 10 to 45% (w/w) as an oil phase, Cremophor EL from 15 to 85% (w/w) as surfactant, and PEG 400 from 0 to 40% (w/w) as cosurfactant at an interval of 5%. A total of the oil, surfactant, and cosurfactant always added up to 100% in each mixture. Percentage transmittance of each combination was recorded. Based on pseudoternary phase diagrams, the oil, surfactant, and cosurfactant were selected and a total of 14 formulations were developed by altering concentrations of oil, surfactant, and cosurfactant by maintaining one soluble adult dose 62.5 mg of bosentan.

Preparation of Bosentan SEDDS

The SEDDS was used for increasing the bioavailability of a drug including various steps: (1) Construction of phase diagram, (2) solubilizing a poorly water-soluble drug and/or pharmaceutical ingredient, in a mixture of surfactant, cosurfactant, and solvent. The oil phase was added to the solubilized drug formulation and thoroughly mixed. (3) The pre emulsion was made as a free-flowing powder by adding microcrystalline cellulose. The powder obtained was filled into size "00" hard-filled gelatin capsules. A series of formulations were prepared with varying concentrations of oil, surfactant, and cosurfactant.

In vitro drug release studies

An *in vitro* drug release study for the optimized formulations was performed using Disso-2000 model dissolution test system (USP apparatus 2), Labindia, India. The dissolution media used for study comprising 900 ml of water and paddle at 50 rpm were used. Preliminary formulations were treated as pre emulsions and were weighed in plastic boats, and the contents were transferred and rinsed with media for complete transfer. All the optimized (FF1-FF5) formulations were converted as dry powder by the addition of microcrystalline cellulose (MCC). About 600–700 mg of MCC was consumed by the formulations and was air dried before filling into the size "00" capsules. An aliquot of the formulation (powder mix equivalent to 62.5 mg of bosentan) in prefilled capsule shell was placed in dissolution media, and the temperature was maintained at 37°C ± 0.5°C. Placebo formulations were also tested to check the interference. 5 ml of samples ($n = 3$) were collected periodically (5, 10, 15, 30, 45, and 60 min), respectively, and analyzed by UV-visible spectrophotometric method at 269 nm. The percentage drug release versus time profiles was studied.

Sedds characterization

Dilution studies[11]

Optimized formulations were subjected to 50-, 100-, and 1000-fold dilution with distilled water, 0.1N hydrochloric acid, pH 4.4 phthalate buffer, and pH 7.4 phosphate buffer. The resultant diluted emulsions were checked manually for any physical changes such as coalescence of droplets, precipitation, or phase separation after 24 h storage.

Optical clarity[2]

Optical clarity was assessed visually as well as spectrophotometrically by measuring the percentage of light transmitted at a wavelength of 300 nm and 600 nm by UV/visible spectrophotometer.

Dispersibility test

As part of self-emulsifying efficiency determination, the efficiency of dispersibility was assessed using a USP apparatus 2. Each formulation (1 ml) was added to 250 ml of distilled water maintained at 37 ± 0.5°C, with paddle rotating at 50 rpm for gentle agitation. The *in vitro* performance of the formulations was visually assessed using the grading system.[11] Grade A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance. Grade B: Rapidly forming, slightly less clear emulsion, having a bluish-white appearance. Grade C: Fine milky emulsion formed within 2 min. Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min). Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface. The formulations that passed the thermodynamic stability and dispersibility tests in Grades A and B were selected for further studies. The tendency to emulsify spontaneously was observed.

Droplet size analysis [20]

50 mg of the **powder formulation** was diluted to 50 ml with distilled water and was allowed to equilibrate for 15 min. Droplet size of the resulting emulsion was then measured by Coulter counter particle size analyzer (Malvern Zetasizer Nano S, Malvern Co., UK). It measures the change in resistance as a function to droplet size.

Table 1: Solubility (mg/ml) of bosentan in various solvents

Ingredient	Solubility (mg/ml) (mean±SD) (*n*=3)
Water	0.02±0.01
0.1N HCl	0.01±0.01
pH 1.2	0.02±0.02
pH 4.4	0.06±0.01
pH 6.8	0.15±0.02
pH 7.2	0.42±0.02
Almond oil	2.03±0.04
Arachis oil	39.46±1.38
Sunflower oil	9.29±0.02
Sesame oil	9.09±0.04
Tween 20	105.37±1.85
Tween 60	77.09±0.95
Tween 80	66.18±0.83
Span 20	15.01±0.57
Span 60	20.16±0.42
Span 80	24.68±0.39
Cremophor EL	78.14±0.76
Gelucire 44/11	116.72±0.92
PEG 400	93.27±1.21
Oleic acid	5.12±0.09
Olive oil	5.52±0.18
Palm oil	6.78±0.07

SD: Standard deviation

Cloud point determination[20]

Cloud point indicates the temperature above which there is a change in the type of emulsion formed. It is the characteristic of a non-ionic surfactant. The cloud point of the formulation should be higher than the body temperature, i.e. 37°C to ensure the stability of the formulation. To measure the cloud point, 1 ml of the formulation was diluted with 250 ml of distilled water, and temperature of the resulting emulsion was gradually increased at increments of 2°C. The temperature at which turbidity appeared was noted down.

Determination of drug content

Bosentan from pre-weighed SEDDS was extracted into 10 ml of methanol:chloroform (80:20 v/v). The extract was then analyzed by spectrophotometry after suitable dilutions at 269 nm.

Refractive index

The refractive indices of the optimized formulations were measured by Abbe's refractometer (Macro Scientific Works, Delhi, India). The refractive index of the formulations was measured at 25°C ± 1°C.

$$Drug\ content\ (\%) = \frac{Actual\ amount\ of\ drug}{Theoretical\ amount\ of\ drug} \times 100$$

Historical data analysis

This design was used for importing data that already exist. This specifies how many mixture and process factors the data were built with. A design expert version of 10.0.5.0 was used for the present study. The study type is a mixture with historical data as design type, randomized as subtype, and design model is linear. A total of five runs with no blocks were used for the study. Three factors such as Gelucire 44/14, Cremophor EL, and PEG 400 were taken and coded as A, B, and C. Optical clarity (%) and cloud points (°C) were taken as responses.

Stability studies

Stability studies of the optimized formulation were carried out at 2–8°C, room temperature, and stability chambers maintained at 40°C ± 2°C/75% and RH ± 5% condition. The samples were withdrawn initially followed by 15 days and 1 month intervals and evaluated for physical stability, drug content, refractive index, and any other instability.

RESULTS AND DISCUSSION

Determination of Solubility

The solubility of bosentan in various solvents (oils, surfactants, buffers, and cosurfactants) studied is given in

Table 1. Solubility study (mg/ml) results obtained revealed that the novel lipid phase Gelucire 44/14 has greater solubility to bosentan 116.72 mg/ml and was selected as a lipid phase for formulation.

Drug-excipient compatibility studies

The FT-IR spectra's of pure bosentan and SEDDS formulation were given in Figure 1 and 2. The peaks were observed at 1110.07/cm due to C-O-C group, a peak at 1168.05/cm due to C-N group, a peak at 1554.28/cm due to C=O group, a peak at 3740.63/cm due -OH group, and a peak at 3395.19/cm due to the presence of aromatic rings in bosentan. This confirms that there is no chemical instability within the formulation.

From the visual assessment studies, there was no change in the characteristics of the formulation during 4 weeks of time span which indicate that there is no physical instability of formulation.

Determination of concentration range of components for the formation of emulsion

All the components were converted into weight/weight percentage before construction of the phase diagram. The darker region in the phase diagram represents the self-emulsification area. Based on the emulsion-forming ability and solubility of drug and optical transparency with oily phase, the surfactant and cosurfactant were selected and

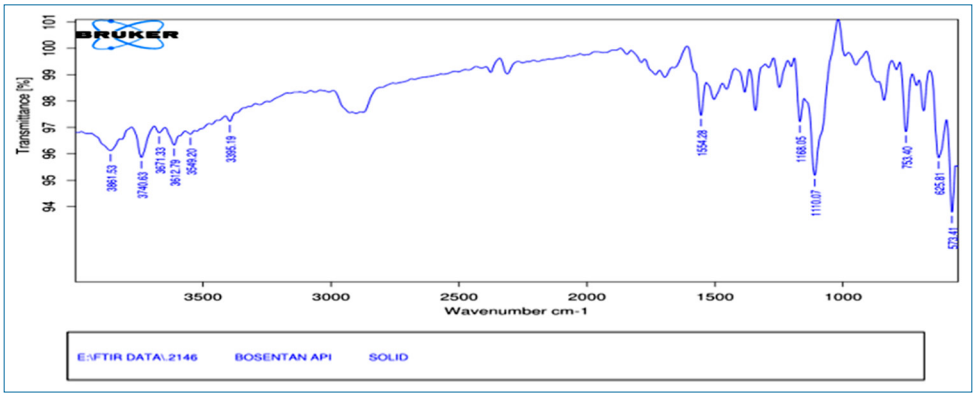

Figure 1: Fourier-transform infrared spectrum of bosentan

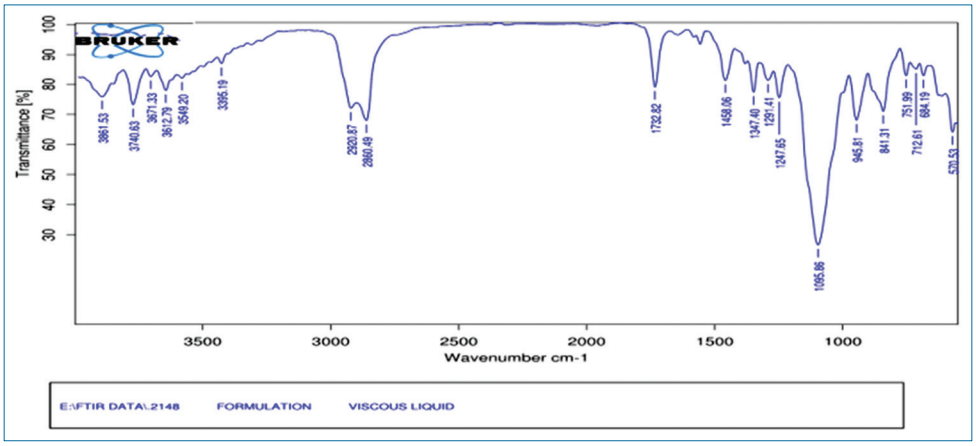

Figure 2: Fourier-transform infrared spectrum of self-emulsifying formulation

Table 2: Emulsifying capacity of selected surfactants and cosurfactants with Gelucire 44/14

Components (1:1)	Number of vortexing require to form emulsion	% Transmittance
Gelucire 44/14:Cremophor EL	3	88.4
Gelucire 44/14:Tween80	7	79.3
Gelucire 44/14:Tween60	6	77.4
Gelucire 44/14:Tween 20	6	78.9
Gelucire 44/14:Span 20	12	80.3
Gelucire 44/14:PEG 400	5	82.2
Gelucire 44/14:PEG 400 (3:1) parts	4	90.3

PEG 400: Polyethylene glycol 400

are given in Table 2. It was observed that the efficiency of emulsification was improved by the aid of Gelucire 44/14 as an oily phase, Cremophor EL as surfactant, and PEG 400 as cosurfactant (Table 4).

Construction of pseudoternary phase diagram

Pseudoternary and ternary phase diagrams were plotted as shown in Figures 3-5. In the phase diagrams, only emulsion points were plotted (shaded area) so that there is no overcrowding of the phases in the diagram, and as for formulation development, only the emulsion region is of interest as given in figures.From Figure 3, it was determined that Smix which contains Cremophor EL and PEG 400 gave greater miscibility zone when used in 1:1 ratio as shown in Figure 6 which was selected for the final formulation, and the ranges of the different components to be used in final formulation were determined by plotting a ternary phase diagrams by mixing the components in various ratios and noting down their % transmittance as shown in Table 3 form the plot shown in Figures 7.

SELECTION OF FORMULATIONS FROM PHASE DIAGRAMS

Based on pseudoternary phase diagrams, the oil, surfactant, and cosurfactant were selected and a total of 14 formulations were developed by altering concentrations of oil, surfactant, and cosurfactant by maintaining one soluble adult dose 62.5 mg of bosentan and the weight of oil, surfactant, and cosurfactant maintained was 500 mg and id tabulated in Table 5.

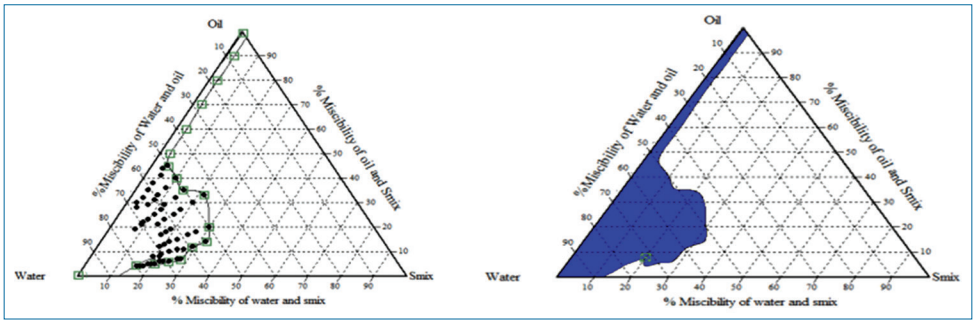

Figure 3: Miscibility points and zone Gelucire 44/14: [Cremophor EL+ polyethylene glycol 400] Smix in 1:1 ratio

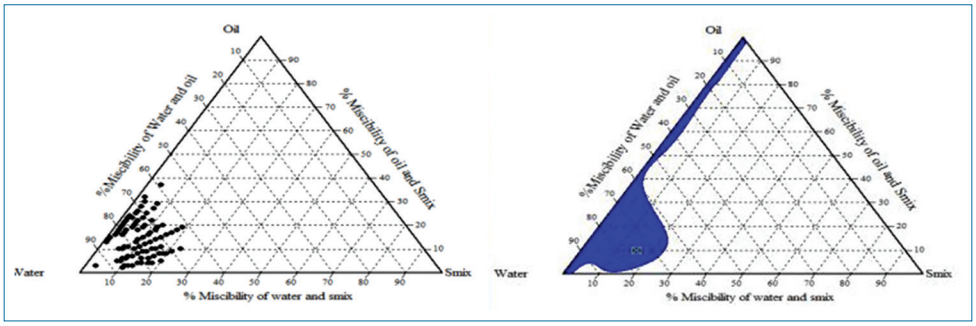

Figure 4: Miscibility points and zone Gelucire 44/14: [Cremophor EL+ polyethylene glycol 400] Smix in 2:1 ratio

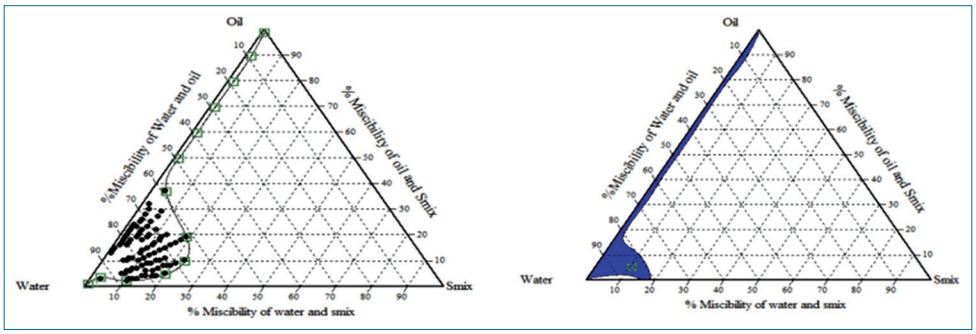

Figure 5: Miscibility points and zone Gelucire 44/14: [Cremophor EL+ polyethylene glycol 400] Smix in 1:2 ratiovv

In vitro drug release studies

In vitro drug release study for all 14 formulations was performed. Percentage release plots are given in Figures 8-10,

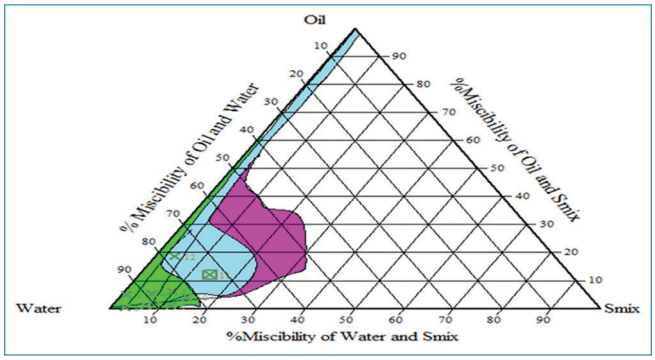

Figure 6: Combined miscibility zones of Gelucire 44/14: [Cremophor EL+ polyethylene glycol 400] Smix

respectively. Optimization was done based on the dissolution profiles of the developed formulations (F1–F14) and the ability of the system to self-emulsify with minimum contact of aqueous phase. Among the formulations, F4, F5, F8, F9, and F13 were selected as final optimized formulations, which shown the highest dissolution rate in water and subjected for final evaluation. The optimized formulations were given the codes as FF1–FF5, respectively, and their composition is shown in Table 6. All the optimized formulations (FF1–FF5) prepared were found to be very stable to dilutions and optically very transparent having small globule size and emulsifies rapidly. The results are given in Tables 8 and 9.

Evaluation of sedds

In the case of dilution studies, no phase separation and precipitations were observed with any media and dilution. All the optimized formulations (FF1–FF5) prepared were found

Table 3: Percentage transmittance values at different concentration ranges

S. No.	Oil % (w/w)	Surfactant % (w/w)	Cosurfactant % (w/w)	% T at 300 nm	S. No.	Oil % (w/w)	Surfactant % (w/w)	Cosurfactant % (w/w)	% T at 300 nm
1	35	65	0	78.8	29	35	45	20	89.8
2	40	60	0	84.8	30	40	40	20	89.6
3	45	55	0	82.8	31	45	35	20	88.3
4	15	80	5	72.6	32	15	60	25	73.3
5	20	75	5	80.1	33	20	55	25	83.8
6	25	70	5	79.4	34	25	50	25	81.2
7	30	65	5	77.4	35	30	45	25	79.5
8	35	60	5	71.4	36	35	40	25	82.8
9	40	55	5	74.8	37	40	35	25	74.6
10	45	50	5	88.3	38	45	30	25	81.5
11	15	75	10	82.2	39	15	55	30	84.1
12	20	70	10	89.2	40	20	50	30	84.2
13	25	65	10	82.2	41	25	45	30	76.8
14	30	60	10	81.8	42	30	40	30	88.2
15	35	55	10	85.1	43	35	35	30	83.6
16	40	50	10	84.7	44	40	30	30	92.9
17	45	45	10	89.8	45	45	25	30	90.2
18	15	70	15	83	46	15	50	35	91.9
19	20	65	15	83.3	47	20	45	35	89.2
20	25	60	15	86.7	48	25	40	35	90.6
21	30	55	15	90.4	49	30	35	35	86.2
22	35	50	15	86.6	50	35	30	35	90.8
23	40	45	15	89.5	51	40	25	35	95.4
24	45	40	15	90.9	52	45	20	35	97.6
25	15	65	20	89	53	15	45	40	93.8
26	20	60	20	89.3	54	20	40	40	94.3
27	25	55	20	89.2	55	25	35	40	59.1
28	30	50	20	89.5	56	30	30	40	62.1

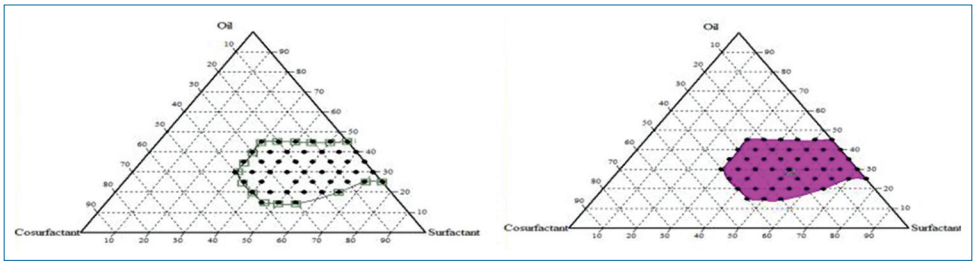

Figure 7: Miscibility points and miscibility zone Gelucire 44/14, Cremophor EL, and polyethylene glycol 400v

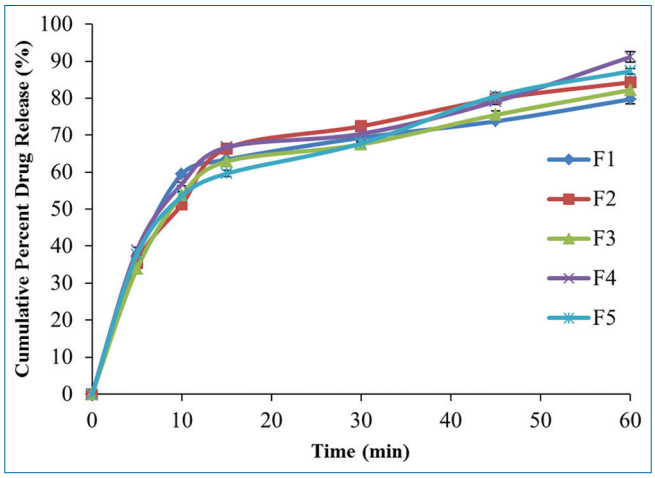

Figure 8: Percentage cumulative drug release versus time (min) F1–F5 formulations

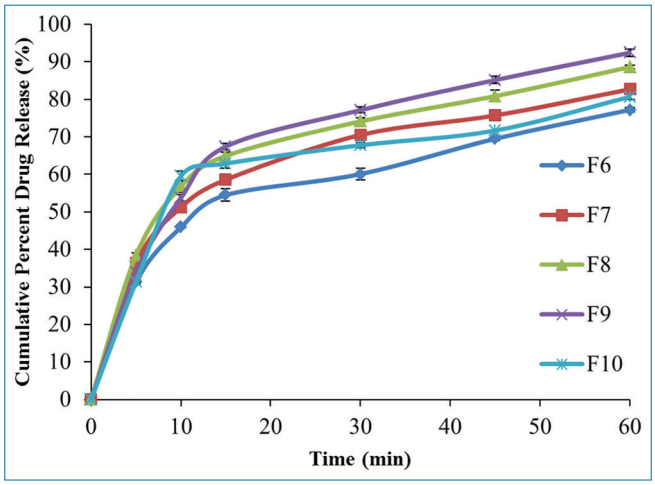

Figure 9: Percentage cumulative drug release versus time (min) F6–F10 formulations

to be very stable and optically very transparent having small globule size and emulsifies rapidly. All the formulations were having the tendency to emulsify spontaneously within 1 min and having a clear appearance. A cloud point result indicates that drug is not prone to precipitation at physiological temperature results as given in Table 7. The initial and final refractive indices did not change by more than 0.05 which indicates that there is no physical and chemical interaction between the components. The refractive index values are given in Table 8. The drug content of optimized formulations (FF1–FF5) was found to be

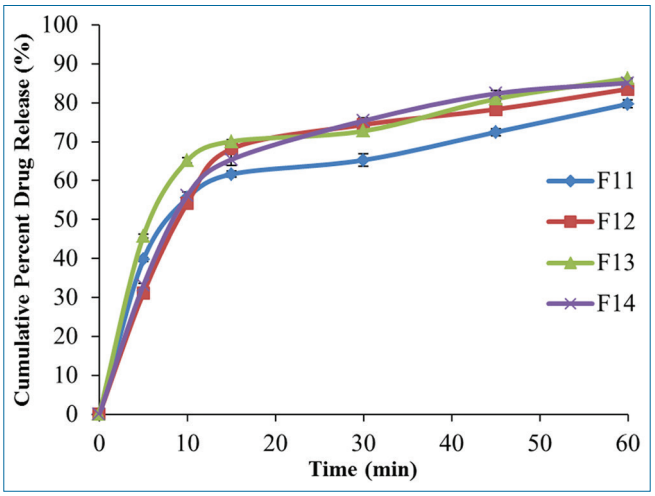

Figure 10: Percentage cumulative drug release versus time (min) F11–F14 formulations

Table 4: Concentration ranges of Gelucire 44/14, Cremophor EL, and PEG 400

Components	Concentration ranges (%)
Gelucire 44/14 (oil)	25–35
Cremophor EL (surfactant)	30–70
PEG 400 (cosurfactant)	25–40

PEG 400: Polyethylene glycol 400

within the limits of the British Pharmacopoeia specifications and is given in Table 9. The mean droplet size of the formulation FF5 was found to be 37.8± 6.2 nm. Hence, the emulsion formed from optimized FF5 formulation was nanosized and this was further confirmed by SEM images of FF5 given in Figure 11. The polydispersity index of FF5 formulation was found to be 0.279 which shows that the globules formed from the SEDDS are of relatively uniform size.

Historical data

Optical clarity

ANOVA for linear mixture model is not significant as the values of "Prob> F" >0.1000. This may be due to no difference in the optical clarity values between the optimized formulations. The three-dimensional (3D) surface plot for optical clarity is given in Figure 12.

Table 5: Formula development of F1–F14 formulations

Formulation code	Drug (mg)	Oil (mg)	Surfactant (mg)	Cosurfactant (mg)
F1	62.5	125	187.5	187.5
F2	62.5	150	175	175
F3	62.5	175	162.5	162.5
F4	62.5	200	150	150
F5	62.5	225	137.5	137.5
F6	62.5	125	175	200
F7	62.5	150	175	175
F8	62.5	175	175	150
F9	62.5	200	175	125
F10	62.5	225	175	100
F11	62.5	125	250	125
F12	62.5	150	225	125
F13	62.5	175	200	125
F14	62.5	225	150	125

Table 6: Optimized formulations of bosentan FF1–FF5

Code	Optimized Code	Oil (mg)	Surfactant (mg)	Cosurfactant (mg)	Drug (mg)
F4	FF1	200	150	150	62.5
F5	FF2	225	137.5	137.5	62.5
F8	FF3	175	175	150	62.5
F9	FF4	200	175	125	62.5
F13	FF5	175	200	125	62.5

Table 7: Optical clarity, dispersibility, cloud point, and drug content of optimized formulations

Optimized code	Oil (mg)	Surfactant (mg)	Cosurfactant (mg)	Drug (mg)	Optical clarity (%T)	Dispersibility Test	Cloud point* (°C)	Drugcontent* (%) (Mean±SD)
FF1	200	150	150	62.5	92.9	Grade A	70±1	103.72±0.41
FF2	225	137.5	137.5	62.5	94.8	Grade A	74±2	102.52±0.21
FF3	175	175	150	62.5	88.2	Grade A	68±1	101.11±0.18
FF4	200	175	125	62.5	91.6	Grade B	70±3	101.24±0.22
FF5	175	200	125	62.5	97.4	Grade A	75±1	102.15±0.16

*n=3. SD: Standard deviation

Table 8: Refractive index of optimized formulations at initial and 1 month time point

Optimized code	Refractive index (without drug)		Refractive index (with drug)	
	Initial	1 month	Initial	1 month
FF1	1.442	1.441	1.511	1.512
FF2	1.449	1.450	1.502	1.503
FF3	1.446	1.446	1.532	1.529
FF4	1.452	1.451	1.521	1.523
FF5	1.448	1.447	1.524	1.521

Final equation in terms of real components:
Optical clarity = +124.20357* Oil+108.63214* Surfactant+29.63214* Cosurfactant
(X1= A: Oil; X2= B: Surfactant; X3= C: Cosurfactant)

Table 9: Stability data in terms of drug content at various storage conditions

Code	2–8°C			Room temperature		40±2°C/75% RH	
	Initial drug content	Drug content after 15 days	Drug content after 1 month	drug content after 15 days	drug content after 1 month	drug content after 15 days	drug content after 1 month
FF1	103.72±0.41	103.69±0.38	103.52±0.42	103.21±0.3	103.53±0.2	102.9±0.4	103.12±0.1
FF2	102.52±0.21	102.48±0.28	102.41±0.15	102.11±0.15	102.21±0.18	102.11±0.3	101.74±0.3
FF3	101.11±0.18	101.09±0.22	100.98±0.28	101.02±0.12	101.02±0.15	100.96±0.3	100.51±0.7
FF4	101.24±0.22	100.79±0.18	100.56±0.11	100.98±0.28	100.67±0.21	101.11±0.2	100.44±0.5
FF5	102.15±0.16	101.06±0.10	100.97±0.12	102.11±0.20	101.97±0.12	101.98±0.1	101.92±0.1

Value expressed as mean±SD, *n*=3. SD: Standard deviation

Cloud point

ANOVA for linear mixture model is not significant as the values of "Prob> F" is >0.1000. This may be due to no difference in the cloud point values between the optimized formulations. The 3D surface plot for cloud point is given in Figure 13.

Final equation in terms of real components:
Cloud Point = +19.00000* Oil+544.00000* Surfactant-576.00000* Cosurfactant-1100.00000* Oil * Surfactant+1700.00000* Oil * Cosurfactant+0.000000* Surfactant * Cosurfactant

(X1= A: Oil; X2= B: Surfactant; X3= C: Cosurfactant)

Point prediction

A point prediction was run to determine the desirability of the system. The concentration ranges selected for optimized formulations are showing desirability close to 1. The desirable concentration ranges were highlighted and shown in the contour plots of Figure 14.

In vitro drug release studies

The drug release studies for optimized SEDDS (FF1-FF5) in comparison to marketed bosentan tablet (Bosentas®) were performed in 900 ml water and paddle at 50 rpm. The percentage drug release was more in formulated bosentan SEDDS than marketed Bosentas tablets in all time points with water. Drug release was observed to be around 10% at 15 minutes time point in case of Bosentas and was between 55-70% in case of optimized formulations. At the end of 60 minutes, almost 90% of the drug was released with optimized formulations and it was only 50% in case of Bosentas. Percentage release plots are given in Figure 15.

Stability studies

The stability data of the optimized formulations FF1-FF5 are given in Table 9 and indicates that the product remained stable at accelerated storage conditions. No color change or spotting reflects that the samples are stable without

Figure 11: Scanning electron microscope image of self-emulsifying drug delivery system of bosentan FF5 formulation

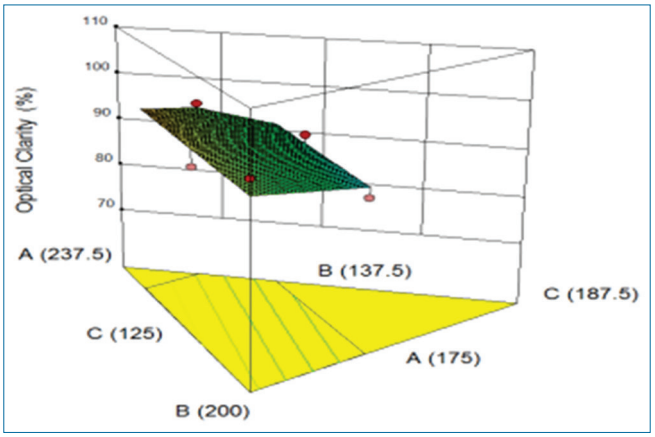

Figure 12: Three-dimensional surface plot for optical clarity

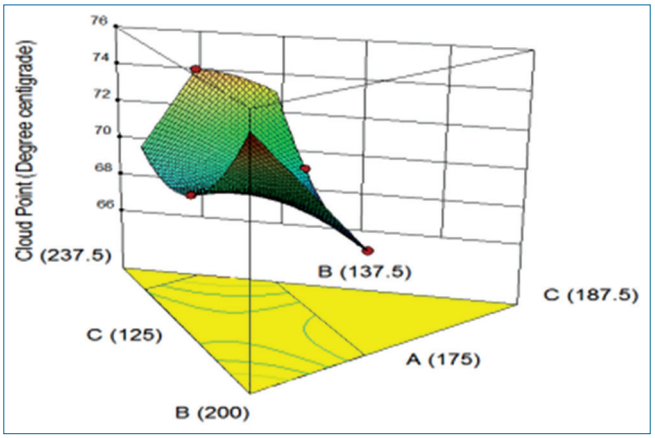

Figure 13: Three-dimensional surface plot for cloud point

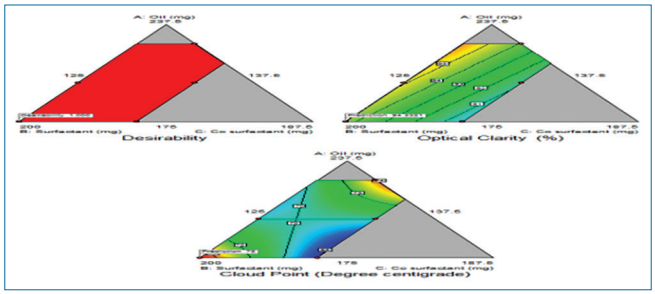

Figure 14: Contour plots for point prediction

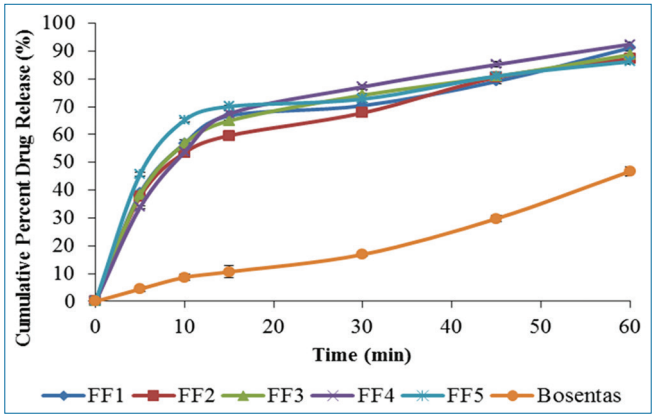

Figure 15: Percentage drug dissolved plot of FF1– FF5 and commercial formulation

any physical degradation after 15 days and 1 month time points.

CONCLUSIONS

Components were selected in which the solubility of bosentan was highest and forms a rapid emulsion on contact with aqueous medium. Physical and chemical compatibility studies indicate that the components used in the formulation of SEDDS were very compatible. Based on the solubility data, bosentan had shown a solubility of 1.0 mg/100 ml in water and 11.672 g/100 ml in Gelucire 44/14 which is miscible in all proportions with water which forms a stable emulsion. Based on miscibility zones obtained from pseudo ternary phase diagrams and further concentration ranges obtained, formulations (F1–F14) of SEDDS were developed. Comparative study of optimized formulations with commercial Bosentas® tablet was perfomed, and the results indicate a tremendous increase in % drug release of bosentan from its convectional Bosentas® tablet. The concentration ranges of oil, surfactant, and co surfactant selected for optimized formulations are showing desirability close to 1. Accelerated stability studies indicate that the optimized formulations were very stable. A faster relief of pulmonary arterial hypertension with effective drug therapy can be achieved using novel technologies like SEDDS for improving patient compliance and faster relief.

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