Bosentan Loaded Solid Self-Micro Emulsifying formulation and evaluation of their In-vitro and Ex-vivo characteristic using Box-Behnken design

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Abstract

The development of the self-micro emulsifying drug delivery system (SMEDDS) of Bosentan is a challenging task. The present work focuses on the development and evaluation of the self-micro emulsifying formulation of Bosentan (BOS). A ternary phase diagram was utilized for the selection of the proper ratio for oil, surfactant, and co-surfactant. An optimized formulation of BOS-SMEDDS composed of Capmul-MCM C-8, Acrysol EL135, Acconon MC8, and Transcutol HP was developed by employing the Box-Behnken design. Optimized liquid SMEDDS was carried out for TEM analysis which shows spherical globule. SEM study confirms that liquid was completely absorbed on adsorbent (Aeroperl 300). The optimized formulation having the capability to self-micro emulsification within less than one minute and droplet size of 116.4 nm (0.41 PDI) was evaluated for In-vitro drug release and ex-vivo diffusion for its comparison with the marketed formulation of the Bosentan.

Key Words: Ternary phase diagram, Box-Behnken, Aeroperl 300, In-Vitro, Ex-vivo

1. INTRODUCTION

Bosentan is a non-peptide, orally active, dual endothelin receptor antagonist is the first endothelin receptor antagonist (ERA) to be used successfully in the treatment of pulmonary artery hypertension (PAH). [1] Bosentan is safe and improves exercise capacity over the short term in patients with Eisenmenger's physiology. [2-3] Bosentan has serious toxicity in the liver, as during PAH treatment with Bosentan liver functional test must be carried out as it increases liver aminotransferase levels. [4-5] Dosing of Bosentan is 62.5mg twice daily up to 4 weeks and then after 125mg twice daily as a maintenance dose so it can cause serious damage to the liver. Bosentan displays dose-and time-dependent pharmacokinetics.

The absolute oral bioavailability of Bosentan in healthy adults is 50% and is unaffected by food. Clearance decreases with increased doses and increases with time. Thus, there is a dose dependency in clearance, which seems to be of limited importance as exposure is proportional to dose in the therapeutic range after oral administration. Upon repeated administration, Bosentan induces its metabolism resulting in a reduction of the AUC of about 35-50%.

Poor bioavailability of any drug is a result of poor aqueous solubility and/or poor gastrointestinal permeability. A top-notch strategy that hits both problems with the same arrow is the lipid-based formulation. Drugs with poor aqueous solubility have usually higher logP value. It means they are nonpolar and more likely to dissolve in oil than water. A special lymphatic pathway for absorption of lipid makes the entry of drug molecules, into blood circulation, along with lipid components easier [6-10]. The problem with a simple lipid-based solution can be resolved by microemulsion. However, microemulsion itself suffers from physical instability. The larger surface area of lipid droplets makes the system thermodynamically unstable

. Self-Micro Emulsifying Drug Delivery System (SMEDDS) is the answer to the problem with microemulsion. SEDDS is a dosage form that produces submicron-sized lipid droplets upon dilution with water or gastric fluid, in the stomach after its ingestion. Formation of spontaneous microemulsion at the site of absorption renders SEDDS a preferable replacement of microemulsion.

2. MATERIALS AND METHODS

Bosentan was procured and provided by Alembic Pharma, Vadodara, India. Different types of oils such as Capmul MCM C8, Capmul MCM, Capmul PG8 and Captex 355 and co-surfactant Acconon MC 8 were gifted from Abitec Corporation, Soyabean oil, Cottonseed oil, Corn oil, Cod liver oil, Linseed oil, and castor oil were purchased from Chemdyes Corporation. Surfactants and co-surfactants like Acrysol EL 135, Acrysol K 140 were gifted from Corel Pharma Chem, Transcutol HP, Gelllucire 44/14 were gifted from Gateffose Corporation, Polysorbate-80, Polysorbate-20, Propylene glycol, and PEG 400 SD fine Chemicals. Aeroperl 300P was gifted from Evonik industries. All other solvents utilized were analytical grade.

2.1 METHODS:

2.1.1 Drug-Excipient Solubility Profiling for Screening of Excipients:

The solubility of Bosentan was carried out by placing an excessive amount of drug into 2 ml of solvent (Oil /Surfactant/Co-surfactant) in 5 ml glass vial with rubber closer. The vial containing the Drug-solvent mixture was subjected to intense sonication for 30 min with heating. The vial was kept unstirred for 48 hours to allow equilibrium in the system. The supernatant was collected and centrifuged at 2000 RPM for 10 min to sediment undissolved drug present if any. 1 ml of post centrifugation supernatant was diluted up to 10 ml with methanol evaluated by UV-Visible spectrophotometric method.

2.1.2 Preliminary Trials for Selection of Combination of Excipients:

Based on the result of drug-solubility profiling and literature review, the first three solvents from each of three categories (oil, surfactant, and co-surfactant) with superior solubility were selected for further study. Combinations (3^3) of oil, surfactant, and co-surfactant give a total of 27 PBE + 27 PBT = 54 preliminary formulation trials (Data not shown). Each trial contains an equal amount of oil, surfactant, and co-surfactant, i.e. 33.33% of each component (1+1+1= 3 ml and 50 mg drug). All preliminary formulations were evaluated for their self-emulsification efficiency and drug precipitation.

2.1.3 Development of Ternary Phase Diagram:

Various points from the ternary plot were selected for the development of the Ternary Phase Diagram. Selected ternary graph points were formulated and evaluated for the rate of self-emulsification and transparency. Formulations with a rate of self-emulsification less than 1

min and transparency of more than 90% were tagged in the ternary plot. The region with self-emulsifying efficiency, thus explored, **was used to determine levels of independent factors in the optimization process** in the later part. A combination of oil, surfactant, and co-surfactant was selected based on the result of preliminary trials and literature review.

2.1.4 Formulation and Optimization of Liquid SMEDDS

The use of Acrysol EL 135 and Acconon MC 8 simultaneously, cause a reduction in individual surfactant requirement (Trials not shown). The same transparency and self-emulsification rate was observed with a low amount of individual surfactants, as found with individual surfactants with a higher amount. So, optimization of all four components, i.e. Capmul MCM C 8, Acrysol EL 135, Acconon MC 8, and Transcutol HP, was performed. Response surface methodology with Box-Behnken Reduction was selected as the design of the experiment. Levels of independent factors were selected from the area of self-emulsification found in the ternary phase diagram.

As a mixture of surfactants reduces the amount of its requirement, in the case of surfactants, a wider range of levels were selected than found in the ternary phase diagram. Detail of Independent factor, Coded and Uncoded levels, dependent factors, and design points are given in Table 1. Checkpoint batches were prepared to evaluate the predictability of the optimization model. The optimized formula was revealed using the Numerical Optimization Tool of the SAS 9.1 Program. Minimum - droplet size (Y1), PDI (Y2), Rate of Emulsification (Y3), Surfactant (X2and X3), Co-surfactant (X_4), and Maximum Oil(X1) were selected asset criteria for optimization.

Each Run point was prepared by mixing respective components in a clean screw-capped plastic tube of 25 ml and mixed thoroughly by vortex mixture. Each formulation contained 150 mg of Bosentan. Tubes were sonicated with heating for 30 minutes and kept unstirred for 24 hours to attain equilibrium.

Table 1: Experimental Design Detail for Optimization of Bosentan Liquid SMEDDS.

Independent Factor	**Coded Level**			**Uncoded Level**		
(Amount in ml)	Low	Medium	High	Low	Medium	High
X_1 = Capmul MCM C8	-1	0	1	2	4	6
X_2 = Acrysol EL135	-1	0	1	2	4	6
X_3 = Acconon MC 8	-1	0	1	1	3.5	6
X_4= Transcutol HP	-1	0	1	1	4	7
Dependent Factors						

Y_1= Mean Droplet Diameter in nm
Y_2= Polydispersibility Index
Y_3= % Rate of self-emulsification in seconds

2.1.5 Solidification of Liquid SMEDDS:

Optimized liquid SMEDDS formulation was converted to free-flowing and compressible powder by absorption with various absorbents or carriers. Three absorbents: Aeroperl 300 Pharma®, Neusiline ULF2®, and Syloid 244 FP® were selected as absorbent. All excipients have a high specific surface area of approximately 300 m^2/gm. Liquid SMEDDS formulation of specified quantity was diluted with 1.5 times more amount of isopropyl alcohol into the mortar. A specified amount of absorbent material was transferred into the same mortar and mixed properly with a spatula until a smooth paste-like mass was produced. This paste-like mass was subjected to a hot air oven at 50^0C for 0.5 to 1 hour until isopropyl alcohol gets evaporated completely. The various proportion of liquid SMEDDS to absorbent material from 1:0.5 to 1:1 (ml: gm.) were investigated. Powder materials prepared by these various proportions are evaluated for Preformulation parameters. The ratio of liquid and absorbent which produce a powder with appropriate flowability and compressibility was selected as the optimum ratio.

2.2 Evaluation:

2.2.1 Self-emulsification efficiency

Self-emulsification efficiency was measured by visual inspection and qualitative grading method described by Charman, W.N [11]. Self-emulsifying Efficiency was estimated using USP-II (USP 30 NF 25) dissolution apparatus. 1 ml of the formulation was added dropwise to 200 ml of 0.1 N HCL (37^0C). The rotating paddle was kept at 60 RPM speed to provide gentle agitation. The rate of emulsification and quality of emulsion after complete dispersion were measured. The time required for complete dispersion: The rate of emulsification, was measured visually using a stopped clock. Emulsion Appearance Qualitative grading (EAQG) of the final emulsion was given according to Table 2.

Table 2: Qualitative Grading Scheme of Final Emulsion after Complete Dispersion.

Emulsion Appearance	Qualitative grade
Rapidly forming (<1 min) with clear or slightly bluish in appearance	A
Rapidly forming (<1 min) with a less clear and bluish-white appearance	B
Forming within 2 min (but more than 1 min) with bright white	C
Takes more than 2 min with dull, greyish white emulsion with	D
Poor emulsification with large oil droplets on the surface of the water	E

2.2.2 Drug Precipitation:

After complete dispersion and determining the rate of emulsification and EAQG, 100 ml of final dispersion or emulsion was transferred in to clean glass beaker and kept aside for visual observation for 24 hours. After 6, 12, and 24 hours bottom of the glass beaker was observed for any sign of drug precipitation against the dark background.

2.2.3 Evaluation of Transparency

Because of fluctuation in NTU in nephelometric analysis, percentage transparency after dilution of SMEDDS formulation with purified water (1 ml with 200 ml) was determined by UV-Visible spectrophotometer at 650 nm [12-13]. For both the drugs purified water was used as blank and standard. Readings were recorded in percentage transmittance (%T).

2.2.4 Droplet Size Determination:

Droplet diameter and distribution (Polydispersibility Index) was measured by Dynamic Light Scattering technique. The sample was prepared by mixing 1 ml of SMEDDS formulation into 200 ml double distilled water with gentle agitation. After one hour sample was subjected to droplet size distribution analysis in Malvern Zetasizer Nano S 90.

2.2.5 Scanning Electron Microscopy:

SEM is used for producing the images of the sample by scanning the sample surface with the help of the focused beam of electrons. For the evaluation of the surface topography of the solid SMEDDS, the SEM was utilized. The sample was coated with gold before the scanning.

2.2.6 Transmission Electron Microscopy

To observe the spherical size and smooth surface of the globules transmission electron microscopy study was performed. Optimize formulation diluted with distilled water. Carbon coated copper grid is used to hold that drop of dispersion followed by drying. To measure the surface morphology transmission electron microscope used in which this grid mounted and pictures captured at a different resolution.

2.2.7 Physicochemical properties for SMEDDS powder

Carr's Index (Compressibility Index) and Hausner's ratio were performed as per USP30 NF25 using USP-I tapped density test apparatus. Accurately weighed 10 grams of powder sample was transferred into a 50 ml graduated measuring cylinder. The angle of repose for estimation of powder flow property was estimated according to the basic method of the angle of repose explained in USP30-NF25.

2.2.8 *In vitro* Dissolution Profile Comparison:

According to the Lipid formulation classification system consortium, this formulation comes under type IIIA/IIIB. Such formulation does not require the digestion of lipids. So, Dissolution media as prescribed in official compendia is selected [14]. Dissolution was performed in 900 ml of 7.4pH phosphate buffer solution. The dissolution test procedure described in USP320-NF25 was employed for the study. Sample collected at each time points were filtered with Whatman® filter paper and subjected to quantitative analysis by UV method of drugs.

2.2.9 Ex-vivo Diffusion Study

Ex-vivo drug diffusion study was performed to prove bioavailability improvement in test product than reference product[15] *Ex-vivo* drug permeation study of Bosentan SMEDDS powders were performed by intestinal sac method [16-19]. Non-everted chick ileum was used for the *Ex-vivo* drug release study. Test and reference samples were prepared by mixing powdered tablets of test or reference in 10 ml of 7.4 pH phosphate buffer. One end of the small specimen of chick ileum (5 cm) was tied with thread and from another end, and either of the test or reference samples was introduced with a syringe. After filling the sample into the chick ileum, another open end was also tied with thread. The intestinal sac thus formed was placed in a glass beaker containing 100 ml of respective media for both drugs with

constant aeration at 37^0C. Constant stirring was allowed with the help of a magnetic stirrer at 100 ± 10 RPMs. 10 ml of the sample was collected at a different time interval and replaced with 10 ml of fresh media. The sample was then analyzed for the amount of drug released using UV- visible spectrophotometric method of Bosentan.

2.2.10 Stability Study:

Accelerated stability study for six consecutive months was performed for both SMEDDS liquid and powder formulation of Bosentan. All products were stored in the same stability chamber, maintained at 45^0C ± 2^0C, and 75% ± 5 RH. Various physical and chemical parameters were evaluated for all products at an interval of one or three (globule size) months.

3. RESULT:

Table 3: Result of Drug Excipients solubility profiling

Oil	Solubility (mg/ml ± SD)	Surfactant Cosurfactant	Solubility (mg/ml ± SD)
Capmul-MCM C-8	**66.4±0.5**	**Acrysol EL135®**	**145.4±5.08**
Capmul-MCM®	**42.25±0.8**	Acrysol K140®	74.5±10.8
Capmul-PG8®	**52±0.5**	Polysorbate-80	34.25±7.3
Captex 355®	2.2±3.07	**Polysorbate-20**	**108.5±6.7**
Soybean oil	6.0±3.33	**Acconon MC 8®**	**168.3±3.5**
Cotton Seed oil	13.3±8.03	**Transcutol-HP®**	**220.15±0.2**
Corn oil	16.2±0.2	Gellucire 44/14®	48.9±2.31
Cod liver oil	17.5±5.02	Propylene glycol	57.4±1.05
Linseed oil	3.8±1.14	PEG-400	69.0±1.25
Castor oil	20.0±1.42		
Lemon oil	14.4±2.01		
Cinnamon oil	14.9±4.22		
Olive oil	15.38±3.4		
Peanut oil	9.0±5.15		
Coconut oil	7.9±5.08		
Peppermint oil	19.3±3.05		

Ternary Phase Diagram:

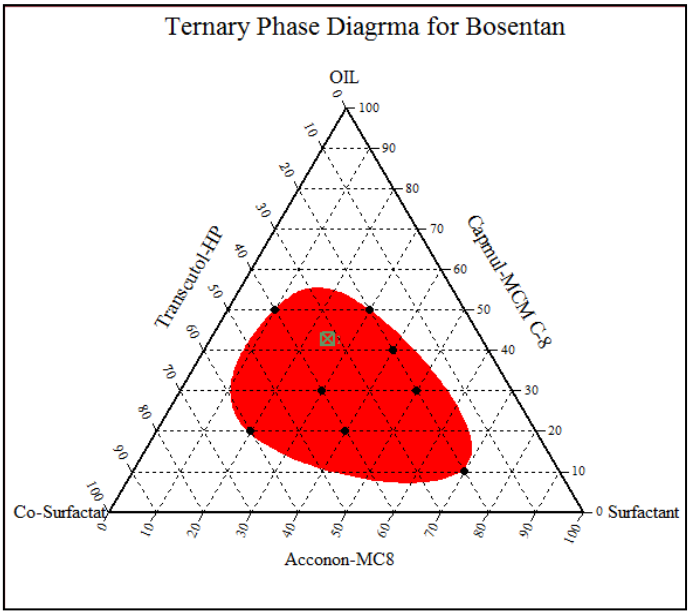

Figure 1: Ternary phase diagram of Capmul MCM C-8, Transcutol-HP, and Acconon- MC8

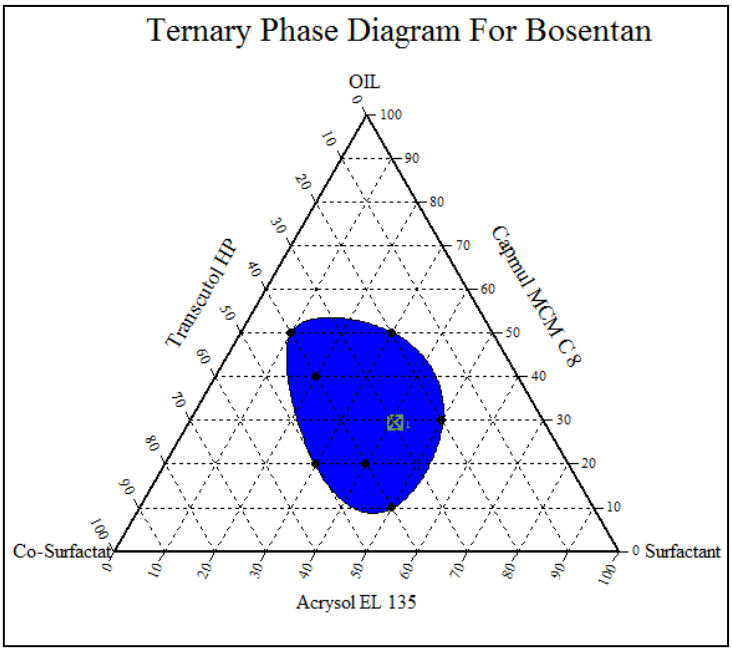

Figure 2: Ternary phase diagram of Capmul MCM C-8, Transcutol-HP and Acrysol EL 135

3.1 Optimization of Liquid SMEDDS of Bosentan:

Table 4: Result of Experimental Design Points (Average[1] ± SD; n=3)

Run	X_1	X_2	X_3	X_4	Y1	Y2	Y3
RBS 1.	0	1	0	1	287.25	0.62	47±1.32
RBS 2.	0	0	1	-1	430.20	0.55	120±0.23
RBS 3.	0	1	-1	0	155.00	0.54	23±0.60
RBS 4.	-1	0	0	-1	114.36	0.69	25±0.65
RBS 5.	1	0	0	1	126.50	0.45	123±0.39
RBS 6.	1	-1	0	0	79.26	0.24	75±0.94
RBS 7.	-1	0	1	0	152.20	0.44	26±0.83
RBS 8.	-1	-1	0	0	70.12	0.47	39±0.27
RBS 9.	0	-1	-1	0	170.20	0.35	45±0.45
RBS 10.	1	0	-1	0	130.78	0.55	39±1.0
RBS 11.	0	-1	1	0	222.25	0.42	50±0.81
RBS 12.	1	0	0	-1	134.26	0.4	122±0.59
RBS 13.	0	0	-1	1	87.80	0.45	63±0.89
RBS 14.	0	0	0	0	209.05	0.29	140±0.21
RBS 15.	0	0	1	1	125.25	0.64	128±0.76
RBS 16.	0	-1	0	1	102.35	0.52	72±2.4
RBS 17.	1	0	1	0	425.90	0.4	142±1.8
RBS 18.	-1	0	0	1	79.25	0.45	51±0.89
RBS 19.	1	1	0	0	418.20	0.75	95±0.27

[1] For Y_1 and Y_2 only Mean found: n=3 formulations were diluted separately and Micro emulsions from three samples were mixed. This mixture of three micro emulsion was analyzed for droplet size.

Run	X_1	X_2	X_3	X_4	Y1	Y2	Y3
RBS 20.	0	1	0	-1	315.20	0.72	54±0.43
RBS 21.	0	1	1	0	499.25	0.75	35±0.78
RBS 22.	-1	1	0	0	113.64	0.78	25±1.4
RBS 23.	0	0	-1	-1	102.20	0.24	50±0.78
RBS 24.	-1	0	-1	0	76.40	0.35	28±1.2
RBS 25.	0	-1	0	-1	98.93	0.25	65±0.56

$$Y_1 = 209.05 + 59.08 * X_1 + 87.12 * X_2 + 94.39 * X_3 + 73.85X_1X_2 + 73.05X_2X_3 - 72.64X_3X_4$$

$$Y_2 = 0.49 + 0.16 * X_2$$

$$Y_3 = 140 + 33.50 * X_1 + 21.08X_3 + 26.25X_1X_3 - 35.50X_12 - 56.13X_22$$

3.2 Regression Analysis:

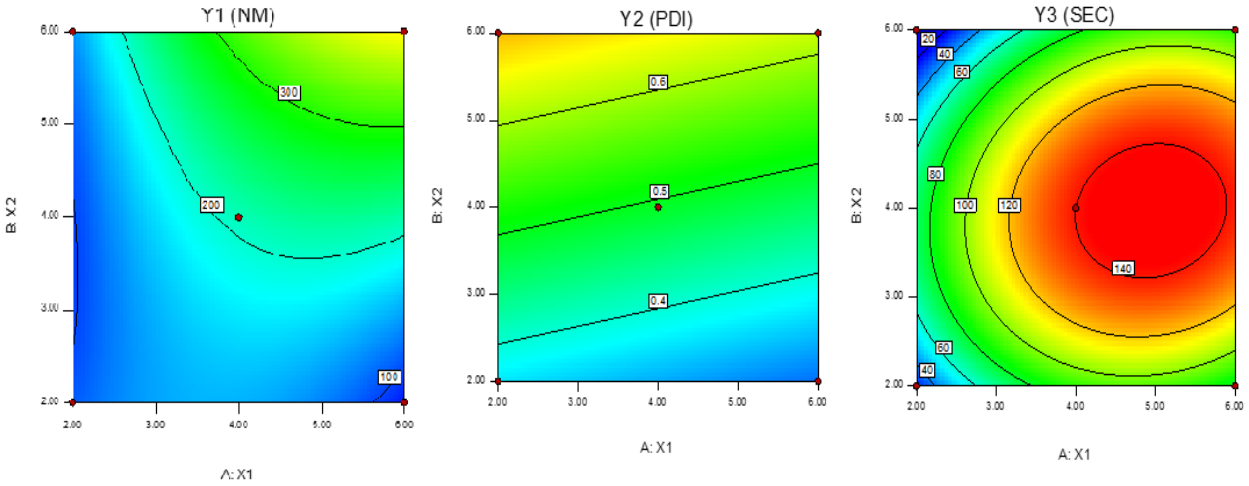

Figure 3: Contour plot for Y1 (Particle size), Y2 (PDI), and Y3 (Emulsification time)

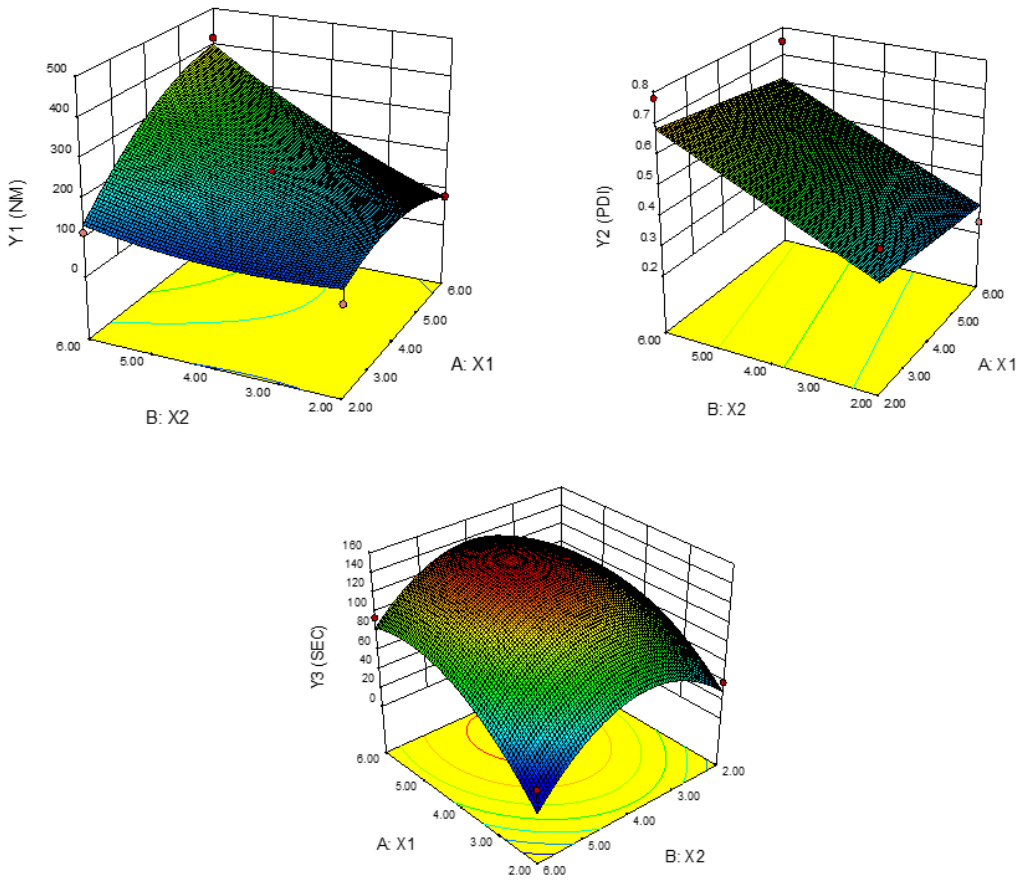

Figure 4: Three-dimensional surface plot for Y1 (Particle size), Y2 (PDI), and Y3 (Emulsification time)

3.3 Result of Check Point Batches:

Table 5: Summary of Check Point Batches Result

Checkpoint batches	Y_1		Y_2		Y_3	
	Predicted	Actual	Predicted	Actual	Predicted	Actual
1	334.82	342.5±0.45	0.59	0.62±0.07	135	145±0.23
2	126	120.7±0.37	0.38	0.48±0.04	82	89±0.56
3	239.38	245.5±0.49	0.41	0.49±0.09	135.2	153.9±0.72
4	140.8	150±0.56	0.56	0.58±0.02	79.04	84.9±0.93

3.4 Optimization of Bosentan Liquid SMEDDS:

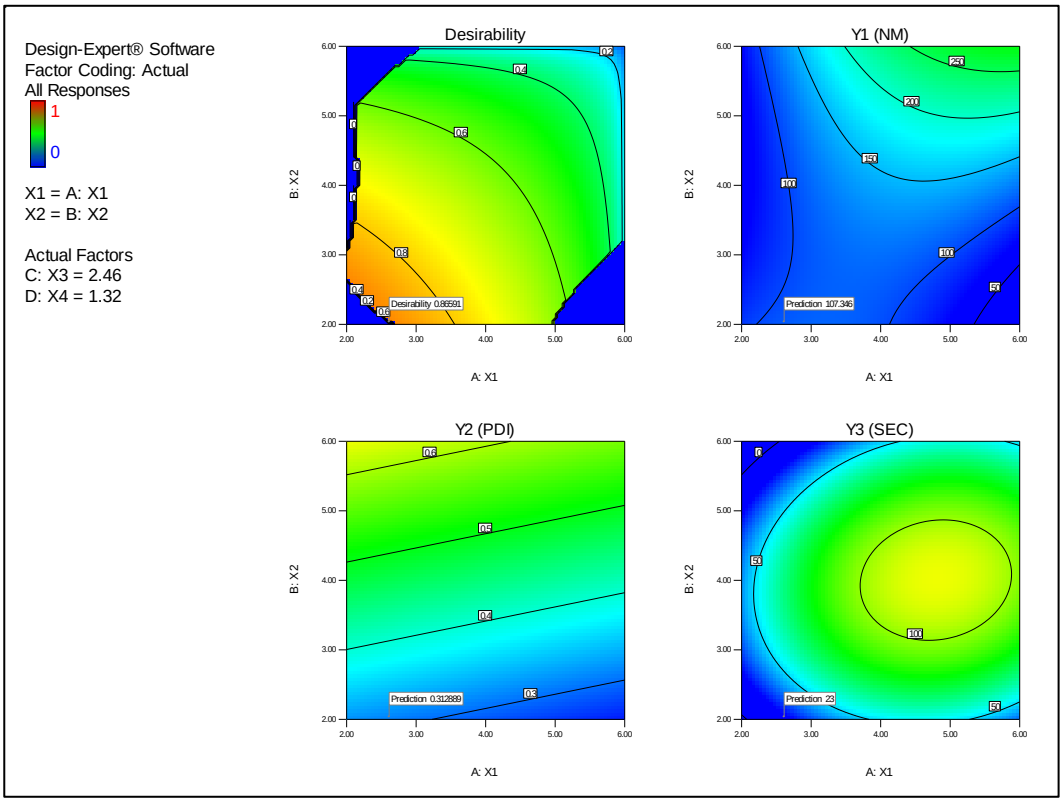

Figure 5: Desirability for Optimization of Liquid SMEDDS of Bosentan

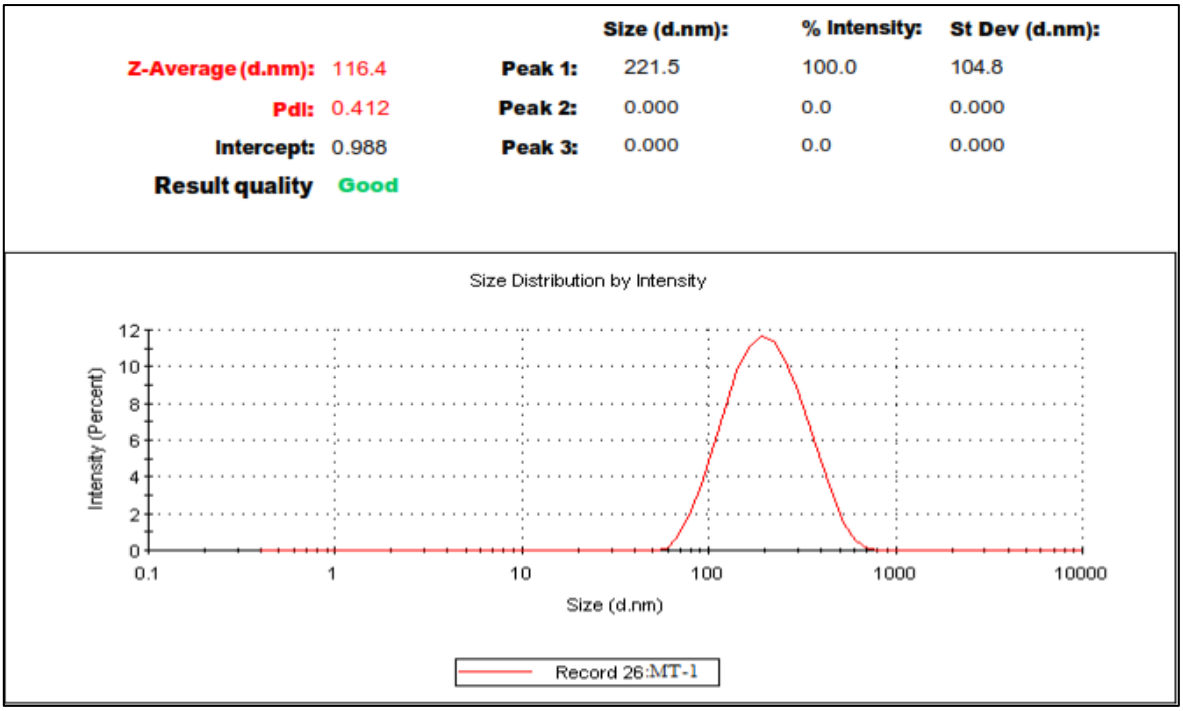

Figure 6: Droplet Size Analysis of Bosentan SMEDDS Liquid Optimized Formulation (MT-1)

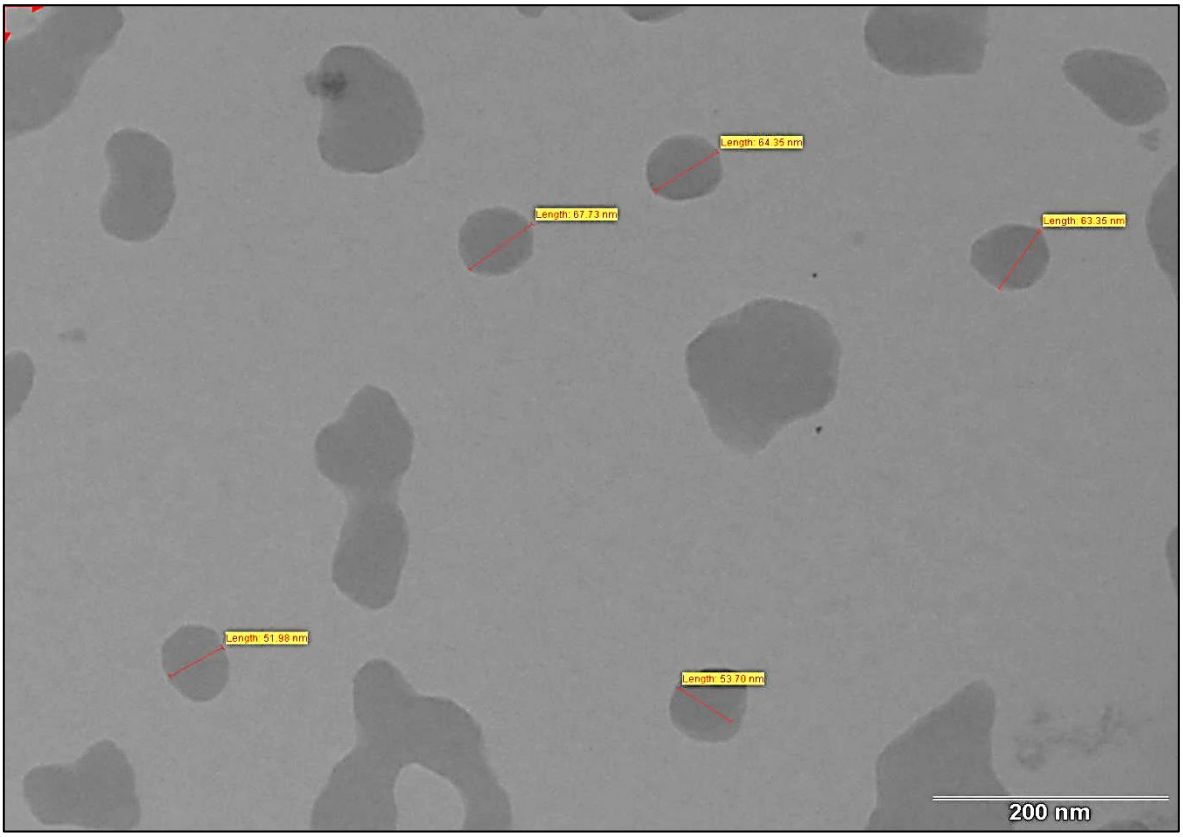

Figure 7: TEM analysis of Liquid SMEDDS of Bosentan (MT-1)

3.5 Solidification of Liquid SMEDDS:

Estimation of Optimum Liquid to Absorbent Ratio:

Table 6: Preformulation Parameters Result for Various Ratio of Liquid: Absorbent

Ratio ml: gm.	Aeroperl 300			Neusilin ULF 2			Syloid 244 FP		
	Carr's Index	Hausner Ratio	Angle of Repose	Carr's Index	Hausner Ratio	Angle of Repose	Carr's Index	Hausner Ratio	Angle of Repose
01:00.5	22.4	1.593	54.8	32.5	1.543	48.8	39.8	1.578	54.6
	±0.12	±0.08	±0.23	±0.67	±0.05	±0.39	±0.39	±0.08	±0.48
01:00.6	19.6	1.425	48.8	29.1	1.378	44.9	36.9	1.489	48.1
	±0.34	±0.08	±0.45	±0.89	±0.16	±0.17	±0.59	±0.18	±0.76
01:00.7	15.5	1.347	39.9	28.8	1.279	40.6	34.8	1.457	45.3
	±0.09	±0.06	±0.98	±0.34	±0.33	±0.03	±0.28	±0.17	±0.91
01:00.8	12.7	1.154	33.8	20.9	1.245	38.8	32.3	1.336	42.5
	±0.56	±0.04	±0.78	±0.12	±0.49	±0.04	±0.02	±0.26	±0.62
01:00.9	13.8	1.125	29.4	18.8	1.189	38.3	29.5	1.387	40.6
	±0.90	±0.08	±0.56	±0.98	±0.28	±0.16	±0.14	±0.08	±0.3
01:01.0	12.6	1.089	28.9	19.5	1.145	39.6	28.8	1.258	38.5
	±0.57	±0.06	±0.45	±0.35	±0.16	±0.78	±0.47	±0.29	±0.17

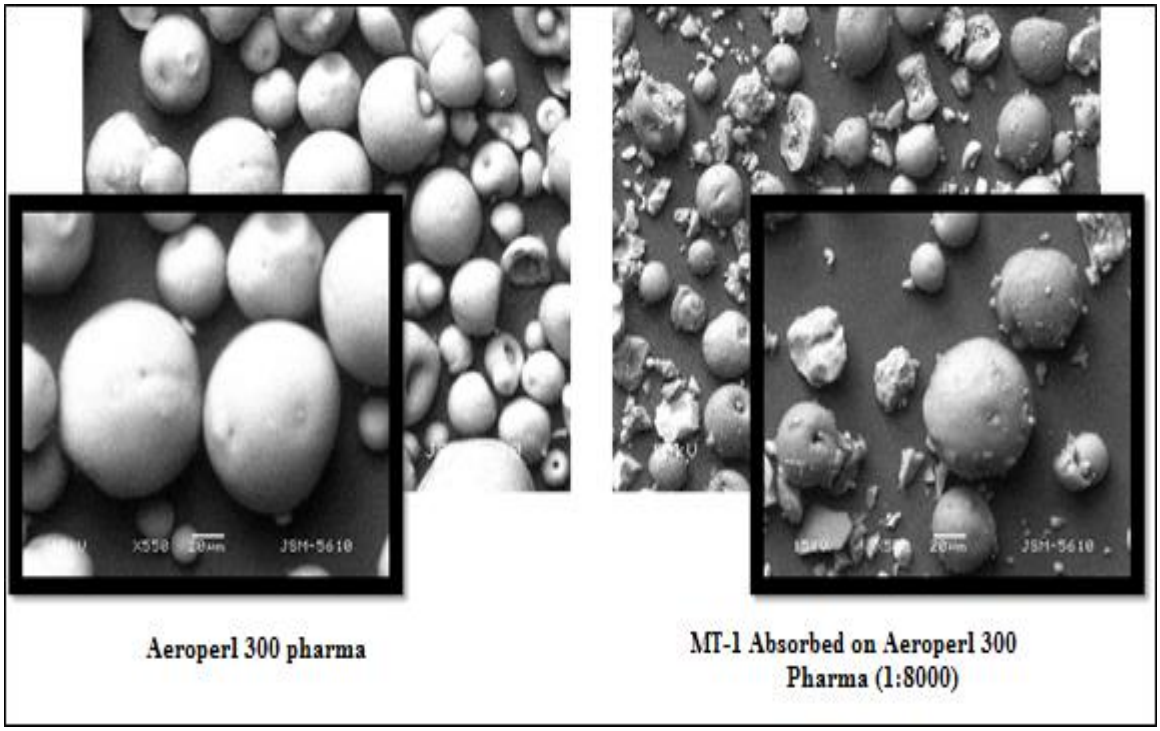

Figure 8: SEM Analysis of Aeroperl 300 Pharma before and after Absorption of Liquid SMEDDS

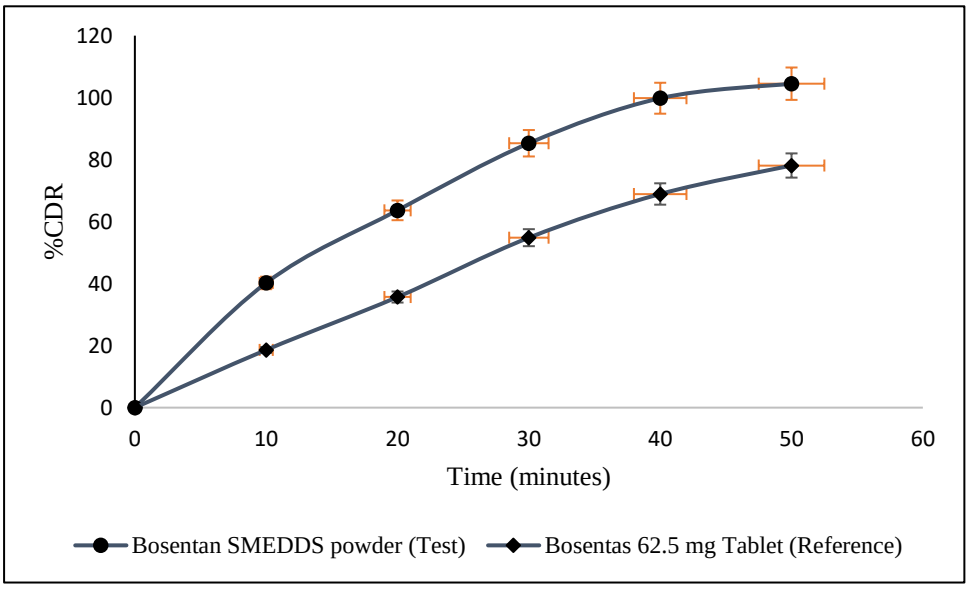

Figure 9: In vitro Dissolution Profile Comparison of Bosentan Products

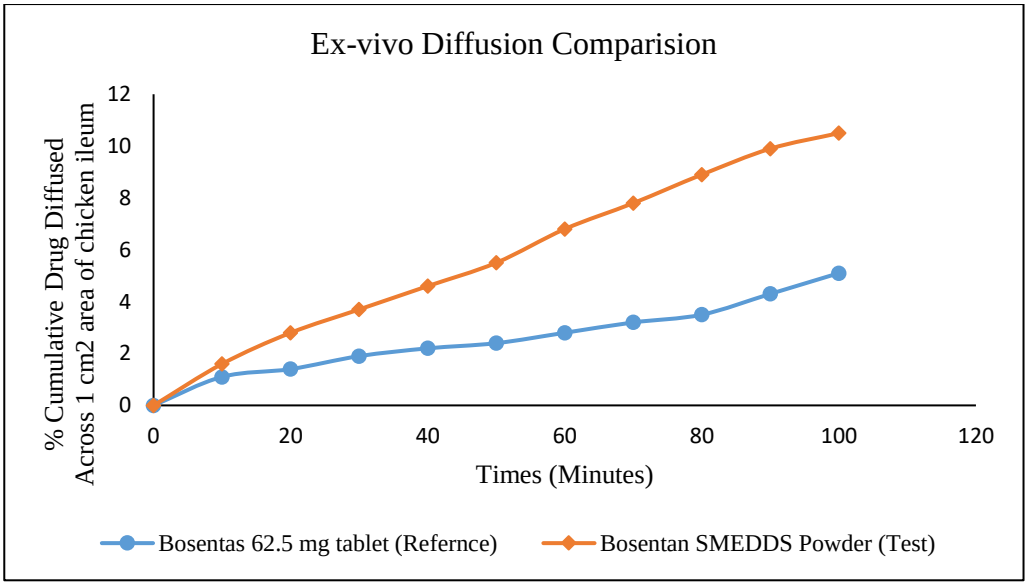

Figure 10: *Ex-vivo* Diffusion Study Comparison between Bosentan Test and Reference Product

3.6 Stability Study:

Table 7: Stability data of optimized formulation of Bosentan

Test Parameters	Acceptance Criteria	Sampling Time in Days (n=3)				
		0	30	60	90	180
MT-1 (Bosentan SMEDDS Liquid)						
Rate of Emulsification (Sec)	NMT 15% variation from initial value	28±1.23	29±1.92	30±1.45	27±0.89	29±1.78
Transparency	NLT 90%	98.78±0.34	97.37±0.79	98.59±0.35	98.89±0.36	97.11±0.48
Drug Precipitation	Not Before 24 hrs	Complied	Complied	Complied	Complied	Complied
Droplet size Analysis (nm)	NMT 15% variation from initial value	116.45±1.93	-	-	121.32±1.25	126.89±1.76
MT-1P (Bosentan SMEDDS Powder)						
Description	White Free flowing powder	Complied	No change	No change	No change	No change
Angle of repose	31-35	33.8±0.78	34.6±0.56	35.1±0.93	34.2±0.18	33.9±0.42
Carr's index	11-15	12.7±0.56	13.1±0.85	12.9±0.23	13.5±0.26	13.9±0.10
Hausner's ratio	1.12-1.18	1.15±0.04	1.16±0.03	1.14±0.07	1.15±0.09	1.16±0.05
% Drug content	NLT 90%	99.34±0.65 %	98.24±0.56 %	97.36±0.34 %	98.38±0.98 %	98.88±0.65 %

4. DISCUSSION:

4.1 Selection of excipients for preliminary trials

Solubility study of Bosentan had screened out various oils, surfactants, and co-surfactants that would be appropriate for further studies. As shown in Table 3, Capmul MCM C8, Capmul PG 8, Capmul MCM, Polysorbate 20, Acconon MC 8, Acrysol EL 135, Propylene glycol, PEG 400, and Transcutol HP were taken into consideration for primary trials. These components were propelled into further studies, due to their satisfactory high solvent capacity for Bosentan. **4.2 Selection of Excipient Combination:**

From the preliminary trials, based on minimum self-emulsification time and precipitation following combination were utilized for preparing the ternary phase diagram.

1) Capmul MCM C8, Acrysol EL 135, and Transcutol HP

2) Capmul MCM C8, Acconon MC 8, and Transcutol HP

4.3 Selection of Levels of Independent Factors for optimization:

The ternary Phase diagram developed for the above-mentioned combinations is shown in Figure 1 and Figure 2. The phase boundary of the ternary phase diagram represents the minimum and maximum value of each component. In the case of Bosentan, due to the synergistic effect of combining Acrysol EL 135 with Acconon MC 8, four independent factors were selected for optimization see Table 1.

4.4 Optimization of Liquid SMEDDS:

Regression analysis Y_1 shows that factors X_1, X_2, and X_3 have a significant effect on globule size. Significant interaction effect over Y_1 was observed by X_1X_2, X_2X_3, and X_3X_4, while X_2 and X_3 show a quadratic effect on globule size. The amount of Acrysol EL 135 (X_2) shows the main and quadratic effect on the polydispersibility index (Y_2). The amount of Capmul MCM C 8 (X_1) and Acconon MC8 (X_3) shows the main quadratic effect on the rate of emulsification. A significant interaction effect over Y_3 was observed by X_1X_3. As per Table-5 results of checkpoint batches validate the entire model. The optimized formula of Bosentan was obtained by Desirability function which is shown in Figure 5.

4.5 Solidification of Optimized Liquid SMEDDS of Bosentan:

As shown in Table 6, the optimum ratio of liquid SMEDDS to Aeroperl 300 having desired Carr's index, Hausner's ratio, and Angle of repose is 1ml to 0.8 gm. Optimized liquid SMEDDS of Bosentan (MT-1) were utilized for the development of SMEDDS powder with

Aeroperl 300. The amount of 3125 mg of SMEDDS powder of Bosentan (62.5 mg) was obtained. It was not possible to formulate a tablet dosage form with such a large amount of Bosentan SMEDDS powders. Bosentan SMEDDS powders can be formulated in the form of a sachet. As per the SEM image shown in Figure 6, it was confirmed that the liquid product was completely absorbed on the solid adsorbent.

4.6 Globule size and TEM analysis:

As per Figure 6, it was observed that optimize liquid SMEDDS shows 116.4 nm of globule size while TEM analysis confirms the spherical shape of globule which was shown in Figure 7.

4.7 *In-vitro* and *Ex-vivo* dissolution study by Comparing with the Market product:

As per the Figure 9 and 10 it was revealed that after 50 minutes marketed product shows 78.11±0.78 % CDR while Bosentan SMEDDS shows 99.81±0.23% drug release within 40 minutes which indicate there was 1.5 fold increase in solubility of Bosentan. In the case of the Ex-vivo study after 100 minutes % cumulative drug diffused across the unit area of chicken, ileum was found to be 10.5 % and 5.1% for test and reference respectively. From the Ex-vivo study, it was confirmed that after converting pure drug into SMEDDS formulation it can easily cross the lipidic membrane.

5. CONCLUSION:

The successful development of Self-micro emulsifying Powder of Bosentan to overcome the issue of their poor bioavailability. The overall conclusion from the entire work is that self – micro emulsifying formulation can be achieved with certain constraints:

1) Dug must fall under BCS Class-II;

2) LogP value must be greater than or equal to 2.5;

3) Dose of the drug must be less than 100 mg;

4) Ratio of dose and solubility into the oil (or SMEDDS formulation) must lie within 0 to 1.

Any drug, which can fulfill the above-mentioned criteria, can be formulated as a self-micro emulsifying dosage form, with justifiable improved bioavailability. It can be delivered in the sachet form by adding flavoring agents. The advantage over tablet dosage form is that low cost and as it avoids disintegration step it can rapidly form Microemulsion as well as rapidly and completely absorbed.

6. CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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