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Formulation and Development of Bosantan Loaded Nanoparticles for Enhancement of its Biopharmaceutical Attributes

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ABSTRACT:

Aim/ Objective: The present work aims to improve the aqueous solubility and oral bioavailability of Bosentan Monohydrate by formulating its solid lipid nano particles which would have Self-nanoemulsifying characteristics.
Methods: The solid lipid nanoparticles (SLNs) were successfully prepared by using emulsification solvent evaporation technique. The optimized SLNs of glyceryl monohydrate loaded with Bosentan monohydrate revealed desired attributes of a optimized solid lipid nano-carrier which had proper compatibility with Isopropyl myristate, Tween 80 and Polyethylene glycol as the lipidic excipients to form stable product. Their optimized concentration ranges resulted in a stable microemulsion region.
Results: Systematic optimization of the liquid SNEDD formulations of Bosentan was performed using in vitro tests and detailed characterization studies. Results revealed F8 formulation produces excellent results with satisfactory results in all the trials of liquid SNEDDS. The optimized liquid SNEDD formulations exhibited globule size less than 100 nm, high and negative values of zeta potential, quick self-emulsification rate, negligible phase separation and high degree of physical stability during thermodynamic evaluation studies. SEM revealed nanostructured particles with negligible aggregation. TEM confirmed the well-defined structure of the nanoparticles
Conclusion: *In vitro* dissolution studies of Bosentan in optimized liquid SNEDDS (F4) unveiled multi-fold enhancement in release profile, as compared to pure API.

Keywords: Bosentan, Solubility, Solid SNEDDS, Lipid System, In Vitro Release

1. INTRODUCTION

Bosentan is used to treat pulmonary hypertension by inhibiting endothelin molecules, which would otherwise cause blood vessel narrowing and high blood pressure [Kaur G et al., 2013]. It's a competitive and selective endothelin receptor antagonist developed from sulfonamides which acquires higher affinity for the endothelin A receptor than the endothelin B receptor. By binding to endothelin, A and endothelin B receptors in the plasmic endothelium and vascular smooth muscle, Bosentan inhibits the action of endothelin-1, a highly potent endogenous vasoconstrictor and Broncho-constrictor which encompasses therapeutic relief in pulmonary arterial hypertension. Both pulmonary and systemic vascular resistance is reduced by Bosentan [Rao SV et al., 2008].

Because of its safety, patient compliance, and ability to self-administer, oral administration remains the preferred mode for drug delivery. Regardless to its advantages, oral delivery has been limited due to several barriers found in the gastro-intestinal (GI) tract [Hauss DJ et al., 200]. The key hurdles observed are low solubility, high first pass metabolism and P-glycoprotein mediated efflux. Hence, insufficient drug dissolution might lead to partial absorption, low bioavailability, and significant variability after oral delivery. For this puspose, maintainace of intrinsic solubilization of the drug inside the GI tract is required for sufficient oral drug absorption. *In vivo* drug precipitation, food interactions and medication properties like sensitivity to degradation, and first-pass metabolism are among secondary factors that might contribute to limited oral bioavailability [Rahman A et al., 2011]. In recent decade, researchers have found that the oral absorption of poorly water-soluble drugs can be improved when given with lipid-rich meals. In contrast to this phenomenon, lipid-based formulations have become popular as a way to improve drug solubility and absorption after oral administration. Lipid-based formulations are thought to be a viable way for lipophilic medicines to improve water solubility and oral absorption [5Khan AW et al., 2012]. The primary purpose of these formulations is to keep the drug in solubilized state in the GI tract. Self-nanoemulsifying drug delivery system (SNEDDS), a versatile carrier will able to improve the solubility and bio-therapeutic activity of many drugs and phytoconstituents. The increased solubility and biopharmaceutical properties of the solid self-nanoemulsifying system containing Bosentan is taken as model drug and it is the prime objective of the study.. It could be hypothesized that Bosentan's biopharmaceutical properties could be improved by including it into SNEDD formulation which maintain the drug in a solubilized state and favors lymphatic circulation [Kalepu S et al., 2013]. As a result, the current study was aimed to develop SNEDDS by utilizing several lipid excipients using oils, surfactants, and co-surfactants, and then spray dried to improve the solubility and stability profile and ultimately form a nano emulsion [Date AA and Nagarsenker MS 2007].

The solid SNEDDS containing Bosentan was developed by spray drying using Mannitol as an inert solid carrier. TEM,Scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray powder diffraction were used to characterize the formulation. The final optimized formulation was evaluated for in vitro drug release studies.

2. MATERIALS AND METHODS

2.1. Materials

Bosentan Monohydrate was obtained from Purechem Pvt. Ltd., Dimethyl formamide (DMF) (Rankem Chemicals, New Delhi), Linoleic acid (Himedia Laboratories Pvt. Ltd.), PEG-400 (Central Drug House, New Delhi), Eucalyptus oil (Sigma Aldrich, USA), Aerosil 200 (Central Drug House, New Delhi), Grapeseed oil (Centaur Drug House, New Delhi), Castor oil (Himedia Laboratories Pvt. Ltd.), Clove oil (Himedia Laboratories Pvt. Ltd.), Ethanol (Rankem

Chemicals, New Delhi), Propylene glycol (Central Drug House, New Delhi), Mannitol (Himedia Laboratories Pvt. Ltd.), Clove oil (Himedia Laboratories Pvt. Ltd.), Lactose (Central Drug House, New Delhi), Methanol (Lobachemie), Microcrystalline cellulose (KunwarLal & Company, Chennai), Oleic acid (Central Drug House, New Delhi), Olive oil (Central Drug House, New Delhi), Potassium dihydrogen orthophosphate (Central Drug House, New Delhi), Sodium chloride (Rankem Chemicals), Sodium hydrogen phosphate (Himedia Laboratories Pvt. Ltd.)

2.2. Solubility Study

Shake flask method was used to determine the solubility of Bosentan Monohydrate in different oils, surfactants, and co-surfactants [Zhao Y et al., 2010]. The drug Bosentan Monohydrate was applied in a vial holding 1 ml of the chosen vehicle, such as oil, surfactant, or co-surfactant. After sealing, the liquid was vortexed for 15 minutes to ensure that the Bosentan monohydrate and the vehicles were properly mixed. After that, the mixtures were swirled on a magnetic stirrer for 45 minutes that was kept at room temperature. After stirring, the solutions were centrifuged at 5000 rpm for 10 minutes, and the medication in the supernatant was tested for Bosentan using a UV-visible spectrophotometric technique at a maximum wavelength of 272 nm [Taha EI et al., 2004]. The linearity equation was used to determine the absorbance values obtained in various oils, surfactants, and co-surfactants. The absorbance data were entered into the linearity equation derived from the Bosentan monohydrate standard curve in acetonitrile, and the quantity was computed [Wang L et al., 2009]. The drug solubility were calculated and expressed in terms of mg/ml by using formula-

Amount= Concentration X **Dilution Factor** X **Volume [1]**

2.4 Emulsification Studies

Emulsification studies are conducted for compatibility with oil phase with different surfactants and co-surfactants. A set quantity of surfactant was selected and required amount of oil phase was added into it. Afterwards, the mixture was evaluated for number of flask inversions and percent transparency. Moreover, amount of oil emulsified was also noted visually

2.5. Liquid SNEDDS Preparation

The ratio of Oil-Smix was used to make SNEDDS (IPM: Tween 80: PEG-400). In a beaker, bosentan was dissolved in surfactant, then co-surfactant and oil were added [Chauhan G et al., 2020]. To obtain a homogeneous mixture, the resulting mixture was swirled continuously with a magnetic stirrer (400-800 rpm) and heated at 40°C [Holm R. 2019]. Because it converts to Emulsion when it comes into contact with water, SNEDDS is also known as emulsion preconcentrate.For the characterisation of nanoemulsions made by reconstituting different batches of SNEDDS, developed formulations were reconstituted in 0.1 N HCl buffer (pH 1.2) and phosphate buffer (pH 7.4) by dropping it in drop-by-drop with stirring until a clear and transparent nanoemulsion formed

2.5.1. Time for self-emulsification

Visual inspection was used to examine the self-emulsifying capabilities of the SLN formulation. 1 ml of each formulation was dropped into 100 ml of each buffer, which was kept at 37°C with gentle agitation at 400 rpm in a magnetic stirrer. The time it took for the emulsion to form (until a clear homogenous system was formed) was visually measured [Shrestha H., 2014].

2.5.2 Determination of Globule Size and Polydispersity Index

Light scattering based on laser diffraction was used to estimate the mean globule size (MGS) and Polydispersity Index of the emulsion using a particle size analyser (Beckman Coulter Counter Delsa Nano C, USA, DLS 4C) [Patidar A., 2010].

2.5.3. Centrifugation test

To create transparent nanoemulsions, optimised preconcentrates were diluted with different media in the ratio of 1:100 v/v with continuous stirring [Chime SA et al., 2013]. The produced nano emulsions were centrifuged at 15,000 rpm for 45 minutes to check for any instability (Ultra Centrifuge, Remi, India). The resulting nanoemulsions were left undisturbed for 48 hours before being examined visually for precipitate development [Thakkar H et al., 2011].

2.5.4. Determination of Zeta Potential

Using the Zeta Sizer Nanoseries, the produced formulations undergo aggregation and electrophoretic mobility. The formulation was poured into 10x10 zeta cuvettes, and particle size and zeta potential were determined with a zeta sizer at a 90° scattering angle. Zeta potential measures the electrostatic charge on the two adjacent particles dispersed in a media. It examines the stability of a nanoemulsion. The small particles exhibit higher zeta potential which showed stability of the formulation. For moderate to excellent stability of the formulation, Zeta potential ranges from ±30 mV to ±60 mV. The developed nanoemulsions were diluted using HPLC water in the ratio of 1:50 v/v and filter using 0.22 µm membrane filters 39 to eliminate multi-scattering phenomenon before analysing. The prepared and filtered diluted sample was then taken into the quartz cuvette for measurement of droplet size, PDI and in capillary cuvette for measurement of zeta potential

2.5.5. Thermodynamic stability test

The physical stability of the liquid preconcentrates was investigated using thermodynamic stress testing. For 7 days of storage, optimised formulations were subjected to heating (50.6 °C) and cooling (60.5 °C) cycles. These formulations were also subjected to a 7-day freeze-thaw cycle at 8.25 °C, followed by heating to 45°C. After that, they were tested for lipid-based instabilities such creaming, immiscibility, phase separation, and so on [Chime SA et al., 2103].

2.5.6. Determination of pH

At pH 4.0 and 7.0, the created formulations were evaluated for pH using a calibrated pH meter.

2.6. Preparation of solid SNEDDS and NLCs

2.6.1 Preparation of solid SNEDDS

Solid SNEDDS of the optimized formulation F4 was prepared using a lab scale spray dryer. Mannitol was selected as a carrier for fabrication of solid SNEDDS. Mannitol (2.14 g) was dissolved in 100 ml of water. Bosentan was added to the optimized liquid SNEDDS to make the final drug content which can ensure the solubilization of Bosentan monohydrate in the formulation as well as preserve the self-emulsifying ability of the formulation. Afterwards, the drug loaded liquid SNEDDS (2.13 g) was dropped into the above-mentioned solution/suspension with continuous stirring at 37℃ for 45 min to obtain homogenous suspension or emulsion [Zasadzinski JA., et al 1997 Plaza-Oliver M., et al 2021]. Then, the aqueous mixture was delivered to the pneumatic nozzle (diameter of 0.7 mm) at a flow of 1 ml/min by a peristaltic pump and a spray dried at inlet temperature of 100°C and outlet temperature of 80°C.

2.6.2 Preparation and Characterization of the developed NLCs

NLCs of linalool have been prepared by the high-speed homogenization method and characterized well for size, surface charge, uniformity, loading and entrapment efficiency and stability studies. The particle size and PDI of the developed NLC was observed to be 184.6±18.28 nm and 0.282±0.071 respectively. This result confirms the nano size range of the carriers with uniform size distribution. At 25°C, NLC dispersion in water has refractive index 1.3328±0.03, viscosity 0.8878±0.076 cP and dielectric constant 78.3±9.4. Smaller PDI reflects uniform size distribution of the particles that is required for better stability during storage of the particles. Nano size range of these carriers is well suited for topical application

2.7. Characterization of Solid SNEDDS

2.7.1. Physiochemical Attributes of Solid SNEDDS

By dissolving 0.1 g of solid SNEDDS in 10 ml of acetonitrile, sonicating it, and filtering it through a 0.22 m filter membrane, the drug concentration of solid SNEDDS was determined. After that, the filtrate was measured using a developed UV method. The samples were placed in a sample microtray and operated at room temperature in a dry assembly mode at a rate of 20 runs per minute. Estimating the time required for dispersion of solid SNEDD formulation in deionized water under stirring and determining the reconstitution rate were used to evaluate the reconstitution properties of optimized solid SNEDDS. The DLS analysis (globule size and zeta potential) of reconstituted microemulsion was performed using the Malvern Particle and Zeta Sizer Analyzer. A UV Spectrophotometer with a maximum wavelength of 540 nm was used to test the optical clarity of the reconstituted microemulsion, and pH was measured with a calibrated pH metre. Several elements in micromeretics investigations, such as specific surface area measurement, were evaluated using the BET Accelerated Surface Area and Porosity System. The angle of repose, tapped density, and bulk density were calculated using the static funnel method and the tapping method, respectively. The compressibility index and Hausner ratio were computed using established methodology.

2.7.2. Instrumental Characterization of Solid SNEDDS

The physical state, compatibility, flow and amorphous behaviour of Bosentan in solid SEDDS were characterized by the differential scanning calorimetry (DSC), FTIR Spectroscopy, Micromeretics study, X-ray Diffraction Study as per standard instrumental parameters and methodologies [Wasan KM., 2001 Dhaval M et al., 2021].

2.8. In-vitro Drug Release Studies

At 37 0.5 °C, drug release experiments from solid SNEDDS were carried out utilising the USP XXIV dissolving apparatus II and 900 cc of phosphate buffer pH 6.8 as a medium. The paddle speed was set to 50 revolutions per minute [Zarif L., et al 2000, Kumar R et al., 2019]. Pure Bosentan powder and Bosentan-loaded solid SNEDDS were packed into 0 size capsules and tested in a dissolving tester. At predetermined intervals, aliquots of 1 ml each were removed. To keep total volume constant and sustain sink conditions, the removed volume was promptly supplied with the same volume of fresh medium. Using the vier dots method at 272 nm, the amount of Bosentan released in the dissolving solvent was measured spectrophotometrically.

3. RESULTS AND DISCUSSION

3.1. Solubility Study

3.1.1 Selection of oil

In the formulation of SNEDDS, the choice of oil is very important as the solubility of the lipophilic drug depends on it. For the self-emulsification, oil is the most important component,

as the absorption of the components can be enhanced through the lymphatics. In the current study, IPM demonstrated highest solubility in drug and selected for further investigations

3.1.2 Selection of surfactant

The chosen surfactant must facilitate emulsification during the formulation development by bringing the interfacial tension down to a very low level, when nano emulsion is developed. In order to give the proper lipophilic property for surfactant, the interfacial area is curved and its stable throughout the entire shelf life. At this moment, non-ionic surfactants were chosen for the experiment because they are known to be less altered by a change in pH. They are also regarded as being secure and biocompatible. Due to their toxicological properties, ionic surfactants were not taken into consideration for the investigation. Non-ionic surfactants were examined in this experiment were tested for emulsification. These substances are typically acceptable for oral administration. According to reports, the non-ionic surfactants have biological activities that cover polysorbates, which have a lymphotropic impact and have an inhibitory effect on P-gp and CYP such as Tween 80.

3.1.3. Selection of co-surfactant

Integration of co-surfactants aids in reducing interfacial tension. It tends to reduce the bending stress at the contact by the hydrocarbon area of the interfacial coating. This eventually leads to an increase in the spontaneity of emulsification. This decreases the polydispersity index and globule size. Additionally, co-surfactants were evaluated based on their compatibility and emulsifying ability with surfactant which was determined by how much oil each could emulsify. Additionally, properly prepared SNEDDS should disperse into nano-sized with gentle agitation, a translucent emulsion forms in a matter of seconds. Based on the preliminary studies, PEG 400 was found to be an ideal co-surfactant for SNEDDS preparation. hence, the conclusion drawn from solubility studies is that Bosentan Monohydrate have observed highest solubility in Isopropyl myristate (oil), Tween 80 (surfactant), PEG 400 (co-surfactant). Table 1 shows the solubility and miscibility of Bosentan Monohydrate in different excipients. Furthermore, emulsification studies were conducted with PEG 400 and evaluation was done on the basis of number of phase inversions and percent transparency (Table 2). PEG 400 displayed significantly higher concentrations of comparative transmittance and emulsification efficacy with less number of phase inversions. Moreover, drug demonstrated good solubility in PEG 400, which was regarded as a unique benefit in in terms of giving the final formulations a larger drug loading. PEG 400 was chosen as a cosurfactant for the following formulations as a result.

Table 1: Solubility data of Bosentan in different lipid excipients

Lipid Excipients	Solubility (mg/ml)	Inference
OILS		
Dimethyl formamide (DMF)	28.58	Miscible
linoleic acid	19.66	Miscible
Coconut oil	6.88	Partially miscible
Clove oil	7.5	Miscible
Grapeseed oil	9.73	Immiscible

Capryol® 90	3.32	Partially miscible
Eucalyptus oil	11.32	Partially miscible
Ethyl oleate	27.92	Miscible
SURFACTANTS		
Quillaja saponin	42.31	Miscible
ECOSURF™ SA9	18.94	Partially miscible
Labrasol®	28.38	Partially miscible
Cremophor EL	22.12	Miscible
Span 60	11.32	Miscible
Propylene glycol	15.32	Partially miscible
CO-SURAFACTANTS		
PEG 400	42.6	Miscible

Table 2: Emulsification study of DMF for co-surfactant selection

Surfactants	No. of Phase inversions	Percent Transparency	Amount of oil emulsified
PEG 400	**11.43**	**97.32**	**3.71**
Propylene glycol	**33.21**	**81.22**	**1.53**
Quillaja saponin	**25.32**	**55.68**	**0.93**
Tween 80	**17.71**	**62.72**	**0.83**

3.3. Characterization of liquid SNEDDS

3.3.1. Self-emulsification Time

The rate of self-emulsification refers to how quickly preconcentrates in the GIT are converted to microemulsion for quick action. It's a metric for evaluating the ability to emulsify in order to make a homogeneous nanoemulsion [Rubin LJ et al., 2002]. The findings of self-emulsification studies on various forms are shown in Table 3. According to observations, Formulation Code (F4) provided the shortest self-emulsification time among the developed formulations. This could be due to a higher rate of spontaneous emulsification and a stronger RHLB correlation with medium chain triglycerides, both of which are beneficial for quick action and therapeutic impact**.**

3.3.2. Determination of Globule Size and Polydispersity Index

Because nano-range micelles have been shown to have a high rate and extent of absorption from the GI tract, globule size and PDI analysis are important in self-microemulsion preconcentrates [Kaur M et al., 2013]. According to the PDI, the formulation was homogenous. Figure shows the results of Bosentan's optimized liquid SNEDD formulations. According to the findings, all of the formulations created nano-structured droplets with little aggregation, but the F4 formulation produced nano-structured droplets with low aggregation (Figure 2). Furthermore, the lower PDI value of the formulation indicated that the observed size was homogeneous, indicating that the formulation was stable. The findings could indicate to a process in which increased surfactant penetration at the oil-water interface disrupts transitory

interfacial tension and shrinks micelles. A modified formulation, according to the data, creates nanoparticulate size with a decreased PDI.

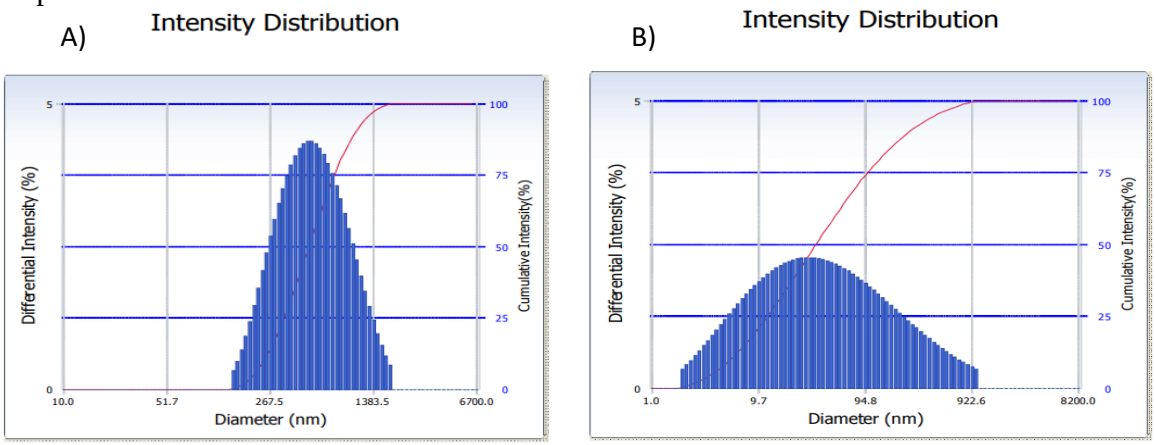

Figure 2: Intensity distribution curve of formulation F4 in: (A) 0.1N HCl and (B) PBS 7.4

3.3.3. Centrifugation test

The test is used to examine the emulsion's stability as well as the generated microemulsion's mono/bi phasic modality. Because the system is kinetically stable, an isotropic system with exceptional stability at a variety of shear rates is required [Rabelli P et al]. Formulations F1, F2, F3, and F4 exhibited no evidence of phase separation in forming emulsions during practical observations, whereas formulations F5, F6, F7, F8, and F9 showed drug precipitation formation, indicating phase separation, and the emulsions were found to be unstable. As a result, in various high shear circumstances, the experimentally constructed selected formulations (F1, F2, F3, and F4) were proven to be physically stable.

3.3.4. Determination of zeta potential

The zeta potential, which depicts the stability of preconcentrates as the absence of globule aggregation in self-forming nano emulsions, is one of the Critical Quality Attributes (CQA) of liquid SNEDD formulation. Several of the developed formulations observed as coagulated and exhibited positive zeta potential values, according to the data. Despite the fact that aggregation and charge were observed to be minor, some of the formulations showed a transient decline in zeta potential values. The effect was more noticeable in the F4 formulation, which has a stronger repulsive attraction than the others (Figure 3). The absence of coalescence plausibility and the emergence of an uncoagulated system are well recognised consequences of the production of stronger repulsive forces in continuous phase with charge on the oil surface [Araz O et al., 2020]. As a result, the F4 formulation was chosen for future research.

3.3.5. pH Determination

The influence of pH on the stability of SNEDDS formulations containing Bosentan was investigated. In all of the dispersion mediums, the self-forming nanoemulsions showed low phase separation and observed with a neutral pH. The results might be indicated that the breakdown of the interfacial coating and generation of stable nanoemulsions could be formed at a neutral pH which was was explained by ionisation of polar head groups of surface-active substances.

Table 3: Complied characterization table of liquid SNEDDS batches of Bosentan Monohydrate

Formulation Code	0.1N HCl					PBS 7.4				
	Particle size (nm)	Zeta potential(mV)	pH	Centrifugation Test	Self-emulsification time (sec)	Particle size (nm)	Zeta potential(mV)	pH	Centrifugation test	Self-emulsification time (sec)
F1	221.5	-11.423	1.35	Passed	15	3214.2	-7.327	6.35	Passed	40
F2	312.7	-9.172	1.82	Passed	29	1548.2	-15.623	6.36	Passed	30
F3	217.4	-7.372	1.52	Passed	36	607.9	-11.387	6.27	Passed	41
F4	423.7	-16.5	1.64	Passed	15	281.3	-29.553	6.54	Passed	45
F5	445.7	-2.177	1.51	Failed	44	284.6	-5.363	6.55	Passed	68
F6	355.3	1.742	1.46	Failed	45	321.8	-9.542	6.62	Failed	65
F7	217.7	2.516	2.81	Failed	60	228.2	1.827	6.63	Failed	95
F8	475.7	-0.565	1.51	Failed	35	389.7	-0.756	6.59	Failed	100
F9	317.5	8.256	1.72	Failed	177	441.7	9.253	6.7	Failed	300

3.3.6. Thermodynamic Stability Test

Because peristaltic activity causes the dispersion of liquid SNEDDS to self-forming nanoemulsion in the GI lumen, it is critical that the generated microemulsion does not behave as partially immiscible or experience phase behaviour changes [Sitbon O et al., 2004]. Liquid SMEDDS are transformed to microemulsion during in situ emulsification and should be thermodynamically stable in the GI lumen. No biphasic regions in the interfacial curvature of microemulsions were seen in appearance after several temperature cycles, indicating that preconcentrate systems (F1, F2, F3, and F4) is stable (Table 4). Tween 80-containing preconcentrates had a greater HLB (14.55) and hydrophilicity, resulting in a more stable o/w microemulsion. Furthermore, high hydrophilicity tends to lower the system's free energy, contributing greatly to the microemulsions stability [Dixit RP et al., 2008]. At the conclusion of this study, no phase separation, creaming, immiscibility, or breaking was seen, notably in

the F4 formulation. As a result, the thermodynamic stability of the chosen nanoemulsion preconcentrate could be assured.

Table 4. Thermodynamic stability test results of developed batches of liquid SNEDDS

Formulation code	0.1 N HCl			PBS 7.4		
	Heating cycle	Cooling cycle	Freeze-thaw	Heating cycle	Cooling cycle	Freeze-thaw
F1	Stable	Stable	Stable	Stable	Stable	Stable
F2	Stable	Stable	Stable	Stable	Stable	Stable
F3	Stable	Stable	Stable	Stable	Stable	Stable
F4	Stable	Stable	Stable	Stable	Stable	Stable
F5	Stable	Unstable	Unstable	Stable	Stable	Stable
F6	Unstable	Unstable	Unstable	Unstable	Stable	Unstable
F7	Unstable	Unstable	Unstable	Unstable	Unstable	Unstable
F8	Unstable	Unstable	Unstable	Stable	Unstable	Unstable
F9	Unstable	Unstable	Unstable	Unstable	Unstable	Unstable

3.3.7. Refractive Index (RI)

To increase the isotropic nature of the nanoemulsion prepared from SNEDDS,refractive index was used.The experimental data of SNEDDS loaded with Bosantan atre summerised in table 5.The range of the refractive index values of SNEDDS loaded with BOSANTAN was found to be between 1.35 ± 0.03 to 1.57 ±0.02 with the optimised value of 1.36 ± 0.03

3.3.8. Percentage Transmittance (%T)

SNEDDS loaded with bosantan were checked for their transmittance values to figure out their isotropic nature.The transmittance values are summerised in Table 5. SNEDDS loaded with Bosantan have the optimum transmittance value of 99.73 ± 0.55.

3.3.9. Percentage Drug Content

Dispersion of drug was found to be uniform which in turn was found out from the percentage drug content mentioned in table 5.The range of percentage drug content was between 99.03 ± 0.64 to100.83 ± 0.42 with optimised value 99.91 ± 0.34.

3.3.10. *Cloud Point (Tc)*

In the case when non ionic surfactants are used, stability is an important factor. Cloud point test is extremely important to depict the stability of SNEDDS and it should be more than 37˚C to avoid phase separation. The cloud point of the optimised batch of SNEDDS loaded with Bosantan was found to be 67.98 ± 2.53 ˚C..Thus the stability of the system can be ensured.

3.3.11. Viscosity

The viscosity of SNEDDS having lower values, may produce o/w type of nano-emulsion depicting their Newtonian flow behaviour.The optimised batch of SNEDDS loaded with Bosantan had viscosity of 300.12 ± 4.69 cps at 25˚C. This outcome points to the fact that the prepared SNEDDS can be easily poured into the capsule and may result in o/w emulsion in-vivo.

Table 5: In house characterizations of developed liquid SNEDDS formulation batches of Bosentan Monohydrate.

Formulation Code	Refractive Index (RI)	Percentage Transmittance (%T)	Percentage Drug Content	Cloud Point (°C)	Viscosity (cPs)
F1	1.63	86.23	91.22	54.42	268.32
F2	1.57	84.41	94.62	36.72	288.84
F3	1.64	94.59	89.32	32.81	256.54
F4	1.26	96.53	99.71	68.78	325.12
F5	1.41	92.32	98.71	54.87	257.42
F6	1.42	95.41	96.41	52.53	433.53
F7	1.75	93.51	97.42	55.75	458.33
F8	1.59	96.72	95.31	55.53	425.64
F9	1.63	85.32	94.42	67.65	439.54

3.4. Characterization of solid SNEDDS

3.4.1. Physicochemical characterization of solid SNEDDS

Spray drying's optimal essential process parameters, such as input and outlet temperature, atomization pressure, and feed flow rate, have had a significant impact. The Bosentan loaded solid SNEDDS generated by spray drying with a hydrophilic adsorbent (Mannitol) were subjected to a variety of characterisation parameters, the findings of which are summarised in Table 6. The appropriate practical yield and Bosentan content in optimised spray dried solid SNEDDS point to homogenous encapsulation employing inert adsorbent [El-Laithy HM et al., 2008]. Micromeritics investigations demonstrated that the solid SNEDDS formulation has good flowability and a higher specific surface area, resulting in improved dissolution and absorption. These findings might be explained by Mannitol's extremely porous character, bigger surface area, and nanoparticulate size, as well as the fact that it has the highest oil adsorption/desorption propensity [Taha EI et al., 2009]. This mechanism favours the possibility of improved flow behaviour, which ensures adequate filling of the capsule dosage form. In terms of surface topography, BET analysis revealed an increased specific surface area of 198.85 m2/g and a large pore volume with a positive Y-intercept of 0.0002010.000024. Reconstitution studies were carried out to ensure the formulation's stability and to imitate in vivo processes in the GI tract [Seo YG et al., 2013]. With the lowest rate of reconstitution, the optical clarity after reconstitution showed higher birefringence and reduced turbidity, implying optimum solid SNEDDS stability. Solid SNEDDS produced small globules with negative electrophoretic mobility, indicating the establishment of a stable nano-colloidal system in terms of globule size and stability.

Table 6. Characterization Parameters of Solid SNEDDS

Study Type	Characterization Parameters	Solid SNEDDS
Physiochemical Properties	Powder Morphology	Free flowing
	Practical Yield	52%
	Particle Size (nm) (powder)	29.759 nm
	Aqueous Solubility	57.5 mg/g
	Specific Surface Area (m^2/g)	188.295 m^2/g
	Angle of Repose	31.11°
	Bulk Density	0.78 g/ml

Micromeretics Properties	Tapped Density Hausner's Ratio Compressibility Index	0.91g/ml 1.26 11.28
Reconstitution Studies	Reconstituted Particle Size (nm) Reconstituted Zeta Potential (nm) pH Reconstituted Rate (seconds) Percent Transmittance (%)	208 nm -13.8 mV 6.98 35 97.1 %

3.4.2. Differential Scanning Calorimetry (DSC)

Bosentan has a pronounced endothermic peak in DSC thermograms between 150 and 165oC, which corresponds to its highly crystalline state and infers its acute melting point. The removal of Bosentan's endothermic peak in the thermograms of solid SNEDDS, however, indicates a full amorphous transition, implying that Bosentan is in a molecularly dispersed state in the formulation (Figure 4).

3.4.3. FTIR spectroscopy

Major stretching and bending vibrations were detected at 3403 cm-1 for the –O-H group, 1350 cm-1 for the –C-O group, 1138.23 cm-1 for the C-C group, 1284.43 cm-1 for the C-N group, 1594.40 cm-1 for the –C=C group, and 2734 cm-1 (symmetric stretch) and 2950 cm-1 (asymmetric stretch) for the –CH3 group, indicating the presence of strong prototype active groups Solid SNEDDS, on the other hand, have widened infrared spectra, which could be owing to partial crystallinity loss or amorphous transformation of Bosentan in the optimized formulation [Xue X et al., 2018] (Figure 5).

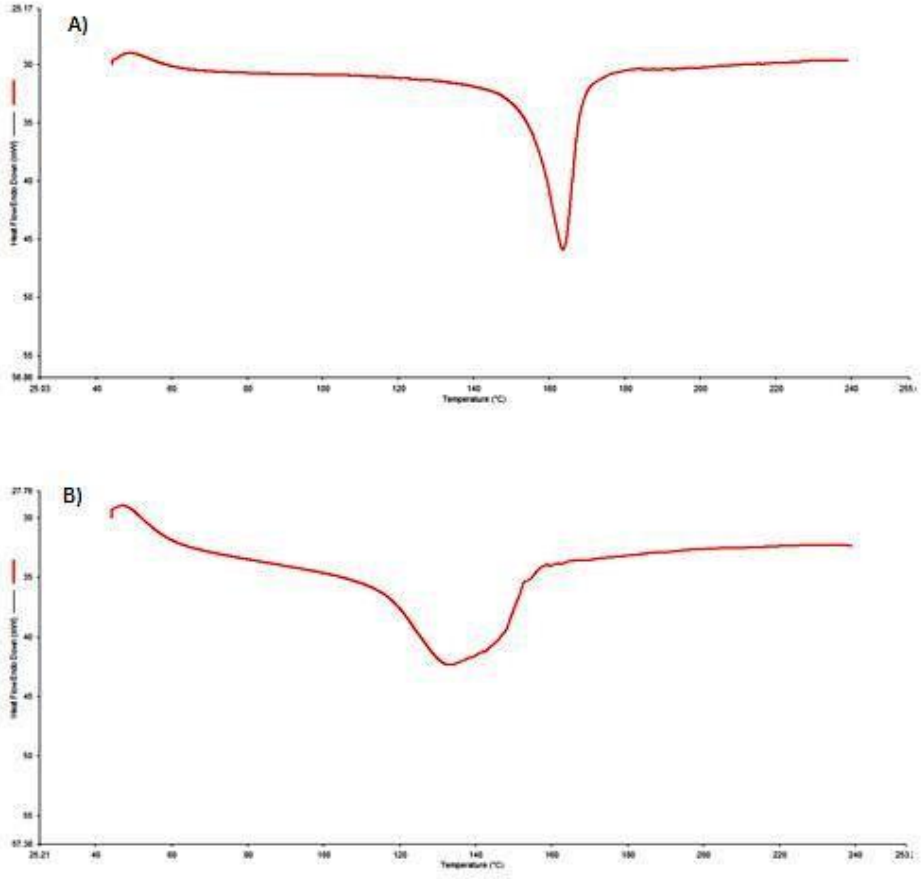

Figure 4: DSC thermograms of (A) Bosentan Monohydrate and (B) Solid SNEDDS

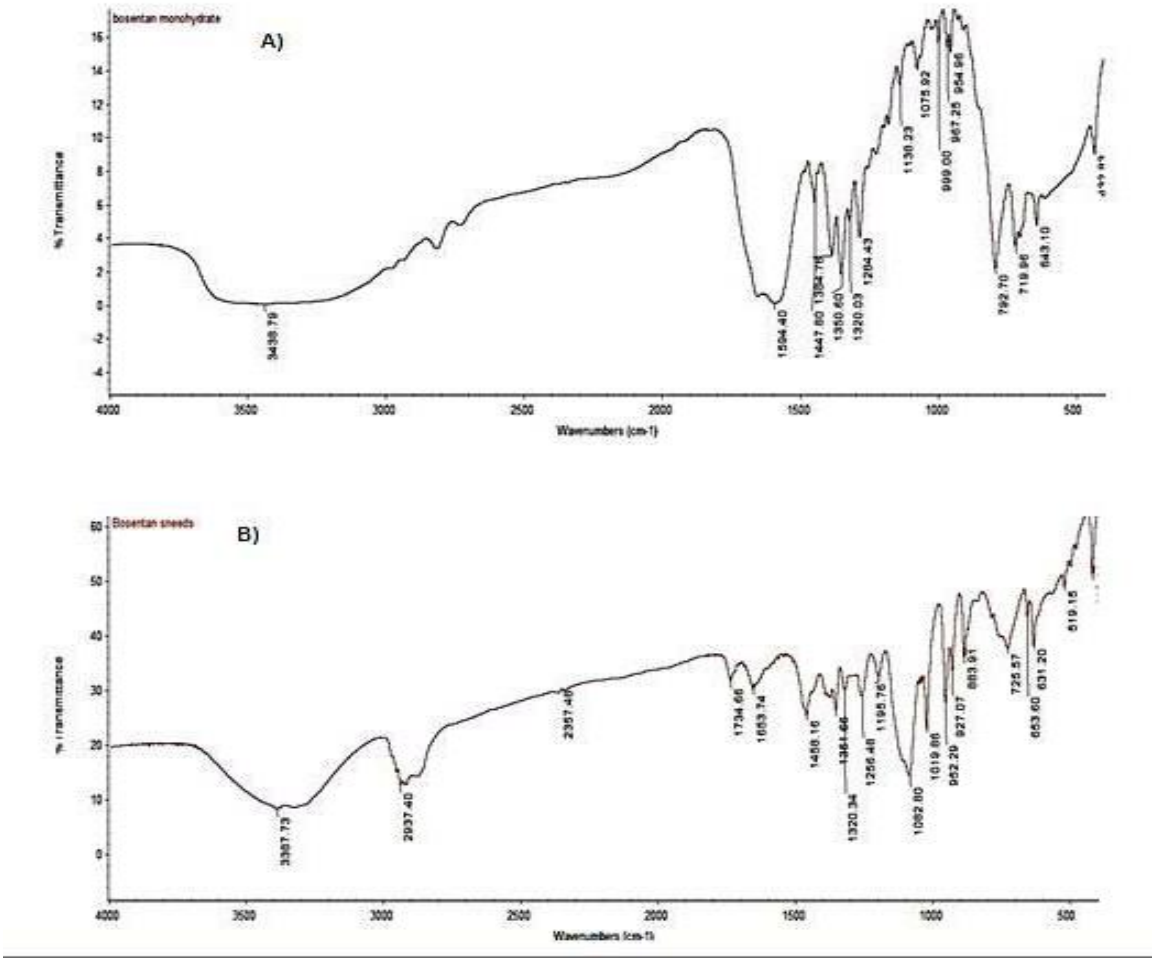

Figure 5: FTIR spectrum of (A) Bosentan and (B) Bosentan loaded solid SNEDDS

3.4.4. Scanning Electron Microscopy (SEM)

Figure shows scanning electron micrographs of Bosentan-loaded solid SNEDDS. Because of spherical particles with smooth outer surfaces and excellent encapsulation of pores, Bosentan loaded solid SNEDDS had a well-defined shape (Figure 6). Furthermore, there were no Bosentan crystals on the surface of the produced solid formulation that showed good encapsulation or were perfectly adsorbed onto the surface of Mannitol. In the current study, these morphological results revealed desired properties of solid SNEDDS incorporating hydrophilic adsorbent.

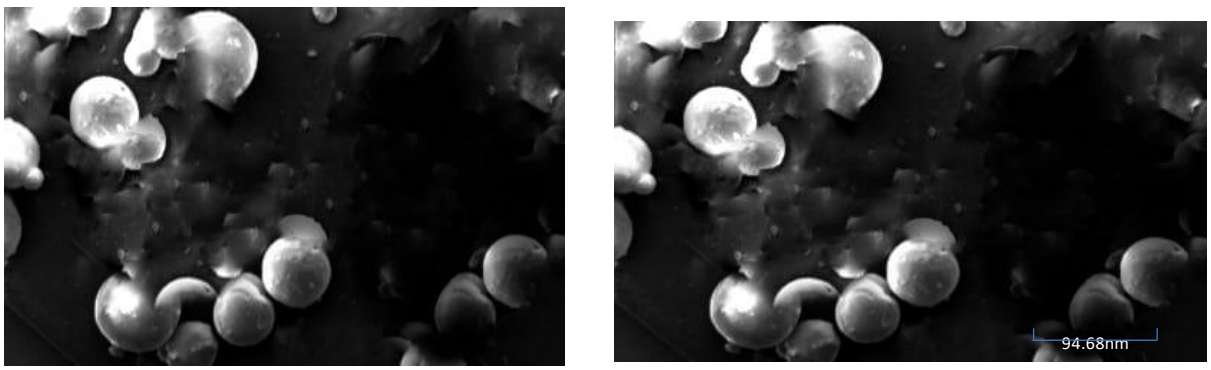

Figure 6: Scanning Electron Monograph (SEM) of Bosentan loaded solid SNEDDS

3.4.5 Transmission Electron Microscopy (TEM)

Morphological characteristics of the SNED was studied using Transmission Electron Microscopy which showed that optimized linalool loaded NLC confirmed that particle was spherical in shape with a well-identified structure. Furthermore, TEM images showed that the uniform size of the NLC detected in the micrographs shows its stability and uniform dispersity. In view of the specimens through the TEM process we can justify that our provided specimen is analogous to the electron flux of reconstituted rate of 35mns. TEM image is given in figure 7.

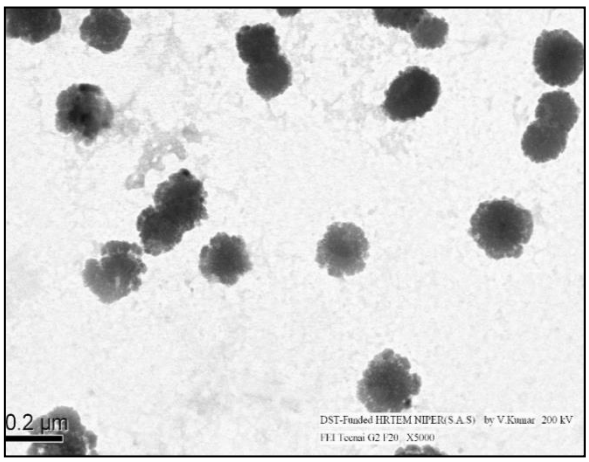

Figure 7: TEM image of SNEEDS

3.4.6. X-RAY Diffraction Study

X Bosentan's X-ray diffractogram typically consists of a random distribution of crystalline solids with sharp diffraction peaks at various 2 degrees of 11.5º, 14.6º, 16.1º, 19.22º, 20.45º, 24.78º, 26.91º, 32.88º, 44.12º, 64.94º and 78.9º, whereas Bosentan in optimised solid SNEDDS showed a reduction in crystallinity (Figure 7).

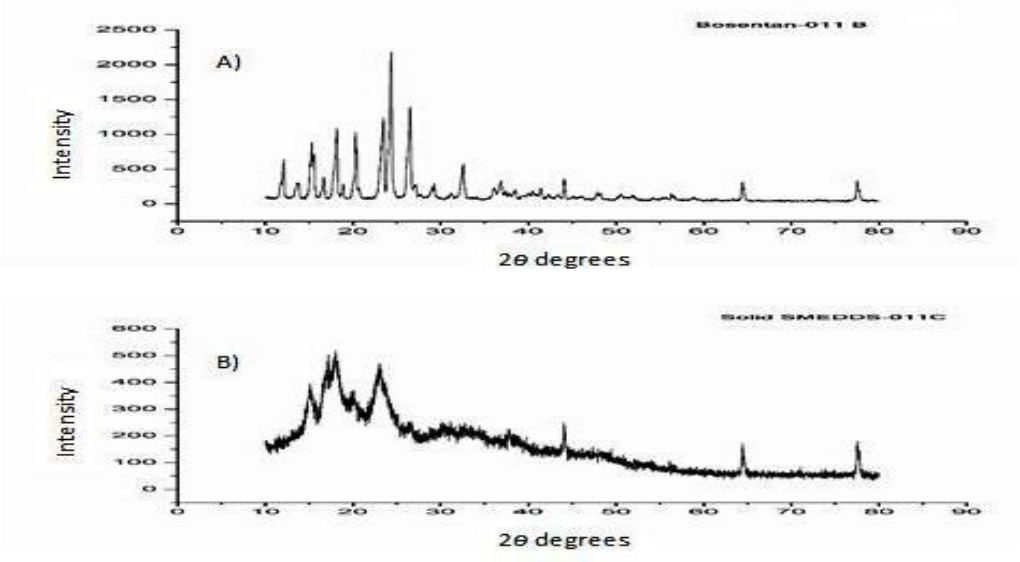

Figure 7: XRD patterns of (A) Bosentan and (B) Bosentan loaded solid nanoparticles administration.

The formed spherical particles having smooth outer surface and effective encapsulation of pores revealed desired characteristics of solid SNEDDS containing hydrophilic adsorbent in the present work

4. *In vitro* drug release study

Dissolution tests were carried in phosphate buffer solution (PBS 6.8) to mimic the in-vivo alkaline conditions in the GI tract, as shown by the intestinal absorption profile of Bosentan. Pure medication has a poor release profile, which could lead to Bosentan's low water solubility and impedes slow drug release in dissolution. In vitro release assays found that Bosentan loaded solid SNEDDS had a burst release effect, with roughly half of the medication being released in 20 minutes. It could be attributable to the Bosentan loaded solid SNEDDS formulation's nano-globular size and rapid self-emulsification rate, which results in instantaneous drug solubilization and a high dissolution rate. Adsorbent hydration happens in the inner core of spray dried particles in the GI lumen. The lipophilic and amorphous domains of drug-rich particles are liberated during hydration, resulting in rapid disintegration. In PBS 6.8pH, solid SNEDDS demonstrated an increase in release profile of 35.85% (Figure 8). Bosentan's enhanced release behaviour in solid SNEDDS formulation is aided by its high surface area, interparticulate void space, and oil's high desorbing capability. Because of its enhanced absorption and bioavailability, this release favours high systemic availability of the medicine in the circulation.

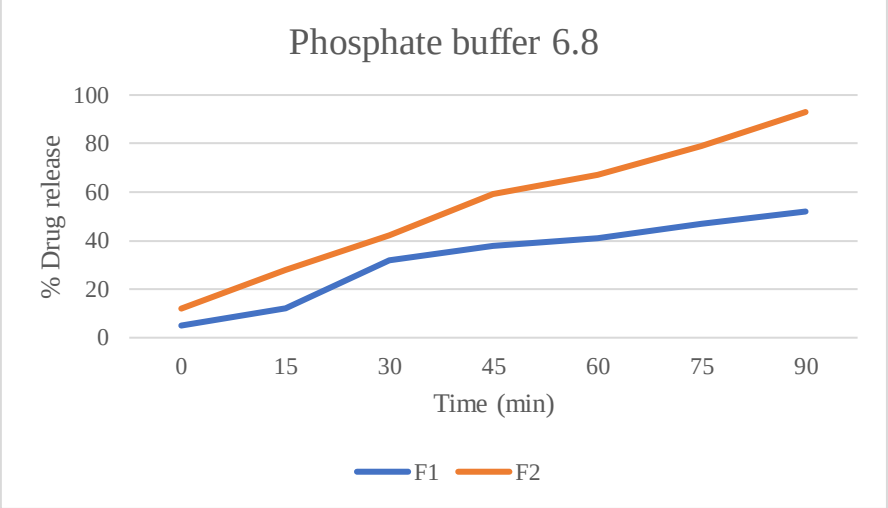

Figure 8: In vitro drug release profile of pure drug Bosentan (F1) and Bosentan loaded solid SNEDDS (F2)

4. CONCLUSION

Various lipid based drug delivery system (LBDDS) have being producing considerable interest from which self-nanoemulsifyng drug delivery system (SNEDDS) play a dominant role in improving the therapeutic profile of lipophilic drugs. The acceptable attributes in developing and optimizing Bosentan loaded solid SNEDD formulation successfully improved its solubility and in vitro profile of Bosentan Monohydrate, as compared to pure API (Active Pharmaceutical Ingredient). The in vitro drug release studies of optimizied formulation showing considerable improvement in dissolution profile of Bosentan Monohydrate. In a nutshell, SNEDDS, a nano-versatile dosage form is now creating interest as they are potential to solubilize the lipophillic drugs and improve the bioavailability and provide future unmet clinical needs for hypertensive patients.

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NIL

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None

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