Research Article

Bosentan-loaded Microemulsion: A Novel Formulation and Evaluation of their *In vitro* and *In vivo* Characteristic

Bhupendra Prajapati[1], Umang Varia[2*]

[1]Department of Pharmaceutics, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar Mehsana-Gozaria Highway, Mehsana-384012, Gujarat, India
[2]Department of Pharmaceutics, Smt. S. M. Shah Pharmacy College, Ahmedabad-Mahemdabad Highway, Aamsaran, Kheda-387130, Gujarat, India

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ABSTRACT

The research's main objective was to improve bosentan's solubility by preparing microemulsion (ME) for pulmonary artery hypertension therapy. Capmul MCM C8 was selected as oil, Tween 20 as a surfactant, and Transcutol HP as a co-surfactant. From the pseudo ternary phase diagram ratio of S_{mix} (1:1) selected. From the ternary diagram's ME area, different batches were prepared, but the drug was precipitate from the formulation, which can be avoided by adding a precipitate inhibitor. Pluronic F 127 was utilized as a precipitate inhibitor in the concentration of 1.5%. The optimized formulation ME 8 contains oil (30% v/v), S_{mix} (60% v/v), and water (10% v/v). The prepared ME was evaluated for globule size 96.71 ± 0.11 nm, % transmittance 99.45 ± 0.54%, and > 99% drug content. Transmission electron microscopy (TEM) confirms the spherical shape of the globule. The physicochemical parameter of ME 8 was performed, and to enhance the stability of ME, it is converted into solid ME by using adsorbent. Aeroperl 300 was selected as an adsorbent in the drug to the adsorbent ratio (1:0.5% w/w) based on physicochemical properties. The *in vitro* drug release investigation after 7 hours % cumulative drug release (CDR) of ME 8 was 78.87 ± 0.17%, and solid microemulsion (SME 3) shows 76.83 ± 0.29%. The pure drug shows only 27.63 ± 0.23% CDR, which indicates that ME revealed better drug release than pure drug. There was a 2.8 fold increase in solubility compare to pure drug. There was a significant change in pharmacokinetics data observed from *in vivo* data compared to the conventional formulation.

INTRODUCTION

Bosentan is a non-peptide, orally active, and dual endothelin receptor antagonist. It is the first endothelin receptor antagonist (ERA) to be used successfully to treat 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 62.5 mg twice daily up to 4 weeks, and thereafter, 125 mg 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 own metabolism resulting in a reduction of the area under curve (AUC) of about 35 to 50%.

To increase the efficiency of already available molecules by designing and developing novel drug delivery systems is a current pharmaceutical field trend. The colloidal system for drug delivery can get success in drug targeting from several drug delivery systems. In the 1940s, Hoar and

*Corresponding Author: Umang Varia (Ph.D. scholar, Ganpat University)
Address: Department of Pharmaceutics, Smt. S. M. Shah Pharmacy College, Ahmedabad-Mahemdabad Highway, Aamsaran, Kheda-387130, Gujarat, India
Email ✉: umangvaria.ph@gmail.com
Tel.: +91-9978787384

Schulman were through light on the concept of a ME.[6] They formulated stable and clear ME by adding an oily phase into an aqueous phase containing surfactants, followed by the addition of alcohol as co-surfactant. Practically in the case of emulsion, although they show excellent stability in terms of kinetic, basically phase separation will observe because of thermodynamic instability.[7] ME is more stable as compared to an emulsion.

Furthermore, the conventional formulation of bosentan available in tablet form, which shows the first-pass effect, leads to decreases in bioavailability. As compared to other formulations, ME shows high drug loading, cost-effectiveness, and stable formulation.

Materials and Methods

Materials

Bosentan was obtained as a gift sample from Alembic Pharma, Baroda, India. Various oils, like Capmul MCM EP, Capmul MCM PG 8 NF, Capmul MCM C8, Captex 355, and Captex 200P obtained as a gift sample from Abitec Pvt. Ltd., Miglyol® 812 from Oleo Chemicals, oleic acid from Chemdyes Corporations and Labrafac PG from Gattefose India Pvt. Ltd. Various surfactants, like Tween 20, Tween 80, Span 80, and Span 20 purchased from Chemdyes Corporations, and Cremophor EL was purchased from Hi-Media Pvt. Ltd. Various co-surfactants, like PEG 400 and polyethylene glycol, were purchased from Chemdyes Corporations. Transcutol HP was gifted from Gattefose India Pvt. Ltd. Various absorbents, like aluminum hydroxide, bentonite, and magnesium hydroxide, were obtained from Chemdyes Corporations. Aeroperl 300 was obtained as a gift sample from Evonik Pvt. Ltd. All the solvents were analytical grade.

Methods

Determination of Bosentan in Rat Plasma by High-Performance Liquid Chromatography (HPLC) Method

Mobile phase preparation: The preparation of the mobile phase for HPLC phosphate buffer of pH 4.6 (6.8 grams of KH_2PO_4 in 1,000 mL) and acetonitrile was used in the ratio (buffer:acetonitrile) 20:80. Mobile phase was filtered through 0.22 µm filter, and then carried out in ultra-sonication for 15 minutes to degas.

Preparation of calibration curve in rat plasma: Weighed accurately 10 mg of bosentan and transferred in a 100 mL volumetric flask to prepare a standard solution. Dissolved the drug by addition of the mobile phase and undissolved part made to solubilize by sonication. An aliquot of 1 mL was withdrawn from the above solution and transferred to the volumetric flask with a capacity of 10 mL. The volume was made with the same mobile phase to prepare a 10 solution. From the 10 µg/mL solution, withdraw 1 mL and dilute up to 10 mL to prepare a 1 µg/mL solution.

Different concentrations (0.1 to 0.9 µg/mL) were prepared, and the samples were inserted into HPLC (Shimadzu LC2010 ATVP) with a manual loop injector. The column utilized for detection was ODS C-18. The ultraviolet (UV)-detector (SPD 10A) at wavelength 273 nm was used. The calibration curve was developed by plotting peak areas *vs.* concentration.

Solubility study of drug in various oil: For the selection of oil, higher amount of bosentan dissolved in various oils (Capmul MCM EP, Miglyol® 812, Capmul MCM PG 8 NF, Capmul MCM C8, oleic acid, Labrafac PG, Captex 355, and Captex 200P), then allowed to solubilize in a bath sonicator for 30 minutes.[8] Further shaking was done in an orbital shaker for 72 hours to form homogenous mixture. After centrifugation, supernatant was collected dissolved in methanol and analyed spectrophoto-metrically at 274 nm.

Screening of surfactants and co-surfactants: Solubility of the drug in surfactant (Tween 20, Tween 80, Cremophor EL, Span 80, and Span 20) and co-surfactant (PEG 400, Transcutol HP, and PG) was carried out by dissolving an excess amount of bosentan in various surfactants and co-surfactants. The bath sonicator was utilized for 30 minutes to get complete solubilization. After that, the shaking was done by orbital shaker for 72 hours. Centrifugation was carried out to collect supernatants. Collected supernatants get dissolved in methanol and analyze UV-visible spectrophotometrically.

Construction of pseudo ternary phase diagram: The pseudo ternary phase diagram is developed to obtain specific ingredients and their concentration range by which a wide ME area can be developed. After selecting suitable components, the ternary phase diagram was developed to get the ME region's nature and extent. For the development of such diagrams, different concentrations of components with a higher number of samples were utilized. Due to the low viscosity and isotropic behavior, the ME area initially delineated. In order to optimize the concentration of oil, surfactant, and co-surfactant, the pseudo ternary phase diagram developed. For that, various combinations of oil, surfactant, and co-surfactant were mixed, in which the ratio of surfactant to co-surfactant varied (S:Co-s), i.e., S_{mix} (1:1, 2:1, and 3:1). To get a wide range of ME region, oil and S_{mix} were mixed in a 1:9 to 9:1 ratio. Pseudo ternary phase diagram prepared by water titration technique and endpoint detection is considered by observing cloudiness or turbidity.[8-9]

Formulation development of ME: From the ternary phase diagram, the ratio of S_{mix} optimized. By trial and error method, various combinations of oil and S_{mix} were selected for the preparation of ME. A fixed amount of bosentan was dissolved in oil, then added S_{mix}, which was then titrated with water to form a clear ME.

Optimization of precipitation inhibitor concentration: To avoid the precipitation of the drug from ME, Pluronic F127

as precipitation inhibitor was added.[10] By addition of precipitate, inhibitor drug loading capability is enhanced. Pluronic F127 was used as a precipitation inhibitor in the concentration varied from 0.5 to 1.5% w/v. Based on drug content, the concentration of precipitation inhibitor selected.

Selection of adsorbent for prepared ME: In order to improve the stability of liquid ME, it was converted into solid form by using adsorbent.[11] Here, aluminum hydroxide, magnesium hydroxide, bentonite, and aeroperl 300 were used to adsorb ME. 1 mL of ME was taken, and adsorbent was added slowly until free-flowing powder developed. The prepared solid ME was then subjected to evaluate their physicochemical properties.

Evaluation

Appearance

All batches were evaluated visually for clarity and any sign of precipitation. Alternatively, under the white and black background, all ME formulations were examined by the naked eye to observe any turbidity sign. The test was performed as per United States pharmacopeia (USP).[12]

Percentage Transmittance

This study shows the product's clarity, in which ME was scanned at 650 nm by UV-spectrophotometer against deionized water as blank. Ideally, ME should be optically clear than conventional emulsion, so as the higher % transmittance indicates good clarity of ME.[12]

Dilutability and Dye Solubility Test

The dye solubility test was performed to observe the type of emulsion, whether it was oil in water (o/w) or water in oil (w/o). The water-soluble dye was sprinkled onto the emulsion surface. If the emulsion is o/w, it is rapidly incorporated with continuous phase, and if the emulsion is w/o than in microscopic examination, clumps of dye are visually indicated. In the dilutability system, dilute with distilled water in 1:10 and 1:100 ratio to observe any sign of separation.[13]

pH Measurement

The pH of the final product depends on the excipient used. From the literature review, it was observed that alteration in pH might fluctuate the zeta potential of the product, which can influence the stability of the formulation. Therefore, pH is also important for the stability of ME. ME's pH value was observed using a digital pH meter standardized using pH 4 and 7 buffers before use.

Viscosity

The viscosity of ME measured by a digital viscometer (Lab Man Scientific Instruments, LMDV 60) confirms the type of emulsion. Higher viscosity shows emulsion is w/o, and less viscosity shows emulsion is o/w.

Globule Size Distribution

The average globule size and polydispersity index of bosentan-ME was measured by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). The sample was diluted with distilled water and then analyzed.

Zeta Potential

To observe the physical stability of ME, zeta potential is an important parameter. In ME, there may be a chance to develop the surface charge on globules. The reason for developing surface charge is a surface group to get ionized, or ions get adsorb. The developed charge further depends on both the surrounding environment of globules and the chemistry of a surface. Around the globules' surface charge create a potential, which is quite high nearer to the surface, and as the distance increase, it gets depleted. In the electric field, the globules' movement can be measured by zeta potential by determining its velocity in the suspending medium. In the present research work, ME was diluted 10 times with distilled water and then analyzed by (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK).

Drug Content

ME equivalent to 10 mg of bosentan was dissolved in methanol (100 mL). The mixture was thoroughly mixed to dissolve the drug in methanol, and absorbance measured by UV-spectrophotometer at 274 nm.

Centrifugation

The purpose of centrifugation was to determine the physical stability of ME. Centrifugation was carried out at 3,000 rpm for 2 hours. After that, ME was examined for clarity, phase separation, and precipitation.

In vitro Drug Release

For the assessment of *in vitro* drug delivery, the dialysis sac method was utilized. The dialysis bag having specified pore size closed with thread from one end to ensure that no leakage of the solution. Drug-loaded ME (2 mL containing drug 62.5 mg) was diluted up to 10 mL with distilled water and mixed for 1-minute.[14] The resultant sample of ME (6.25 mg/mL) was introduced into a dialysis bag and the other end closed tightly with thread. Take a glass beaker containing 100 mL of phosphate buffer having pH 7.4 at 37 ± 2°C. A magnetic bead stirrer managed the beaker's agitation, and aluminum foil was utilized to cover a beaker to avoid solvent loss during the study. At specified time intervals, 5 mL aliquots withdraw from the dissolution medium, and 5 mL of fresh aliquots are introduced, which maintain the same experimental condition.

In vivo Drug Release Study

A single oral dose *in vivo* drug release study was carried out for an optimized batch of ME 8. For that, Wistar albino rats (male) were selected, having weight 250 ± 20 grams. To

determine the plasma level time profile of the drug, the rat is considered the most feasible animal. The Institutional Animal Ethics Committee (IAEC) approved experiments of the Rofel Shri G. M. Bilakhia College of Pharmacy; protocol no. ROFEL/IAEC/2018/4. The first group was given the suspension of bosentan and the second group received the bosentan-ME dispersion. The blood sample was collected at a predetermined time interval into heparinized tubes from the orbital sinus. The plasma separated immediately by cooling centrifuge (REMI) at 5,000 rpm for 15 minutes, and plasma stored at -20°C until analysis. Drug presence in rat plasma measured using HPLC. The plasma concentrations at different time intervals were evaluated using Win Lin software.

Extraction Technique of Bosentan from Rat Plasma

The protein denaturation technique carried out the extraction of bosentan from rat plasma (250 µL). Plasma consisting of various concentrations of the drug was collected in the Teflon lined cap centrifuge tube, followed by 2 mL of acetonitrile. A vortex shaker properly mixed the prepared mixture for 2 minutes. The organic phase was separated by centrifugation at 3,300 rpm and followed by filtration of organic layer by 0.2 µm membrane filter. The collected filtrate (20 µL) was then injected into the column, and the eluents were analyzed at 273 nm. The extraction procedure was performed for all the concentrations.

Physicochemical Parameters of Solid Microemulsion (SME)

After converting into solid form, ME was investigated for its physic-chemical parameters, like the angle of repose, Carr's index, Hausner's ratio, bulk density, and tapped density. These parameters indicate flow behavior of prepared solid ME.

Characterization of Optimized Batch

Transmission Electron Microscopy (TEM)

To identify the spherical size and smooth surface of globules, a TEM study was performed. Optimized formulation diluted with distilled water. Carbon coated copper grid used to hold that drop of emulsion. To measure the surface morphology, TEM was used, in which this grid mounted and pictures captured at a different resolution.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR study of the optimized batch was performed to investigate product stability, as well as, the interaction of the drug with used excipients occur or not. FTIR spectra of bosentan were related to the spectra of bosentan and polymer. Loss of bosentan peaks or fluctuating of the peak in each of the spectra were considered.

Stability Study

The main aim of stability is to prove how the aspect of the drug changes with time beneath the effect of various natural factors, like humidity, heat, and light. Further, it is helpful to form a re-analysis period and shelf life for the formulation, as well as, prescribed storage conditions. Stability tests should examine those aspects of the pharmaceutical formulation prone to change at the time of storage and are likely to affect quality, potency, and safety. For that, samples were subjected to vials and kept at 40 ± 2°C and 75 ± 5% RH, using a stability chamber. Stability study was done by parameters of solid ME like its physicochemical properties, appearance, and drug content.

RESULT AND DISCUSSION

Calibration Curve of Bosentan in Rat Plasma

The calibration curve of bosentan in rat plasma showed in Fig. 1 with linearity in the concentration range from 0.1 to 1 µg/mL. The correlation coefficient 0.9968 was observed, and the peak area of various concentrations was depicted in Table 1. The drug's retention time was observed at 3.89 minutes, which was described in HPLC spectra (Fig. 2).

Solubility of Bosentan in various Oils

The solubility of the drug in various oil depicted in Fig. 3, like Capmul MCM EP, Miglyol 812, Capmul MCM PG 8 NF, oleic acid, Labrafac PG, Captex 355, Capmul MCM C8, and Captex 200P. In this study, Capmul MCM C8 shows the highest solubility 65.4 ± 0.87 mg/mL, which was selected for further
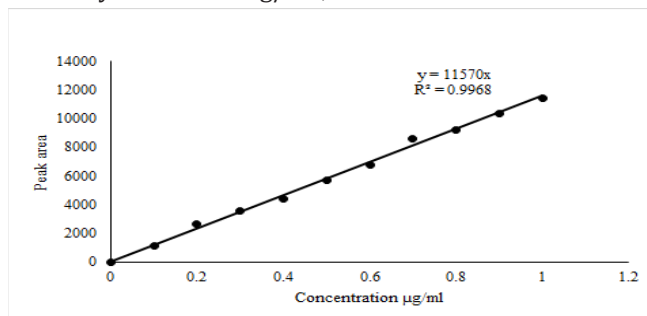

Fig. 1: Calibration curve of bosentan in rat plasma

Table 1: Peak area of bosentan for calibration curve in rat plasma

Concentration (µg/mL)	Peak area[*]
0	0
0.1	1,135.78 ± 0.08
0.2	2,656.89 ± 0.01
0.3	3,534.76 ± 0.05
0.4	4,398.40 ± 0.04
0.5	5,687.43 ± 0.09
0.6	6,798.34 ± 0.02
0.7	8,589.54 ± 0.06
0.8	9,190.56 ± 0.04
0.9	10,378.9 ± 0.06
1	11,451.8 ± 0.03

[*]Mean ± SD; n = 3

investigation because Capmul MCM C8 was composed of mono and diglycerides of medium-chain fatty acid, which acts as an excellent solvent for many organic compounds.

Screening of Surfactants and Co-Surfactants

Solubility of the drug in surfactant (Tween 20, Tween 80, Cremophor EL, Span 80 and Span 20) and co-surfactant (PEG 400, Transcutol HP, and PG) were depicted in Fig. 4. From
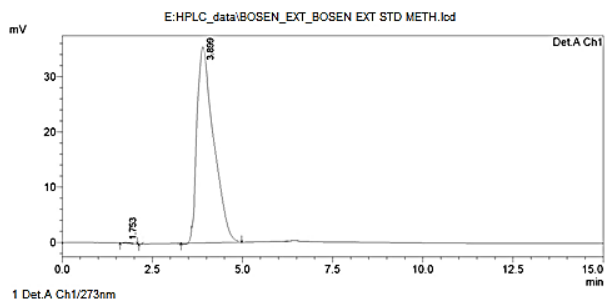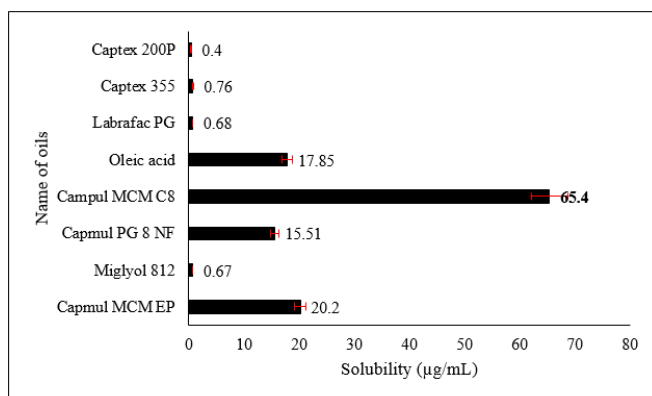

Fig. 2: HPLC spectra of bosentan at 273 nm

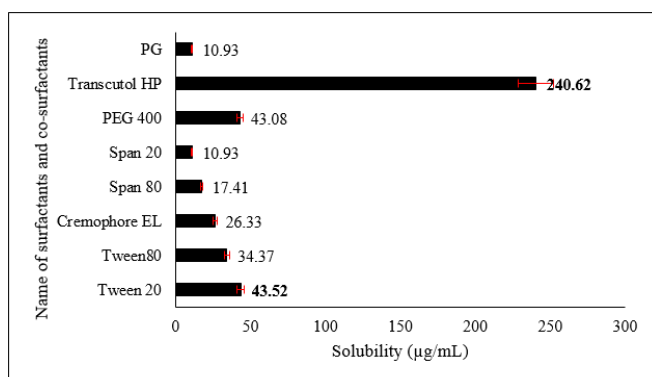**Fig. 3:** Solubility of bosentan in oils

Fig. 4: Solubility of bosentan in surfactants and co-surfactants

Table 2: Composition of ME formulations

Batch No.	Drug (mg/mL)	Oil (% v/v)	S_{mix} (% v/v)	Water (% v/v)
ME 1	31.25	20	50	30
ME 2	31.25	30	60	10
ME 3	31.25	40	50	10
ME 4	31.25	50	30	20
ME 5	31.25	60	30	10

the different surfactant and co-surfactants, Tween 20 and Transcutol HP were selected as they show the highest solubility 43.52 ± 0.31 and 240.62 ± 0.78, respectively. Furthermore, Tween 20 works best as a solubilizer that binds water and oil together. It also acts as a viscosity modifier, which helps prevent changes to the product's viscosity when subjected to varying room temperature. Transcutol HP is a diethyl glycol monoethyl ether, a powerful solubilizer, and is used as co-solvent in oral surfactant formulations.

Pseudo Ternary Phase Diagram

To get a stable ME with exact concentration range, pseudo ternary phase diagram was constructed by the water titration method. Tween 20 as a surfactant and co-surfactant Transcutol HP in the ratio of 1:1, 2:1, and 3:1 were used to prepare the diagram. The zone of ME was obtained, which is shown in Fig. 5. A larger area of 1:1 was selected, and from that region, five formulations were taken randomly to get optimized batch. Tween 20 has high hydrophilic lipophilic balance (HLB) value than Transcutol HP, the concentration of Tween 20 increases incorporation of water can be increased, but the solubility of drug decreases; therefore, 1:1 selected for further studies. From the selected ternary phase diagram 1:1 ratio of S_{mix}, five different batches of ME were prepared by trial and error method. The composition is described in Table 2.

Evaluation

Appearance of ME

As per data shown in Table 3, initially, all formulation shows a turbid appearance because of precipitation of drug from the formulation. Drug content observed from 69.43 ± 0.12% to 80.56 ± 0.24%, and % transmittance was found in the range of 54.67 ± 0.32% to 87.67 ± 0.93%. ME 2 shows a good % transmittance with highest drug content. To avoid precipitation, Pluronic F127 was added as a precipitate inhibitor in different concentrations. After the addition of Pluronic F127 as precipitate inhibitor, there was no sign of precipitation, and formulation remains clear.

Optimization of Concentration of Precipitate Inhibitor

After addition of Pluronic F127 in ME2 ranging from 0.5 to 1.5%, there was a little precipitation in ME 6 and ME 7, while in case of ME 8 with 1.5% of Pluronic F127, there was no precipitation. This is because of increasing the
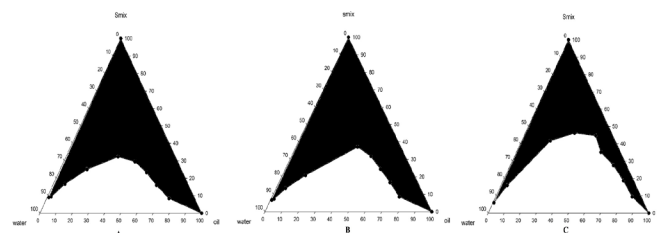

Fig. 5: Pseudo ternary phase diagram of S:Co-s ratio **(A)** 1:1; **(B)** 2:1; **(C)** 3:1

Table 3: % transmittance and drug content of trial batch

Batch No.	Oil (% v/v)	S_{mix} (% v/v)	Water (% v/v)	% transmittance[*]	% drug content[*]
ME 1	20	50	30	54.67 ± 0.32	69.43 ± 0.12
ME 2	30	60	10	87.67 ± 0.93	80.56 ± 0.24
ME 3	40	50	10	82.45 ± 0.91	72.12 ± 0.98
ME 4	50	30	20	83.45 ± 0.26	75.87 ± 0.53
ME 5	60	30	10	85.98 ± 0.46	77.58 ± 0.19

[*]Mean ± SD; n = 3

Table 4: Optimization of concentration of precipitate inhibitor

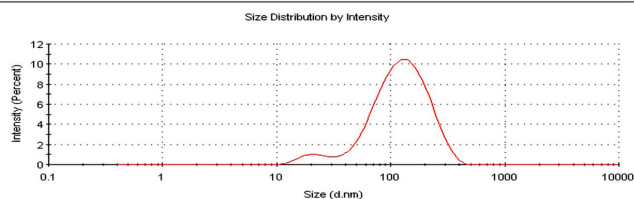| Batch No.	Oil (% v/v)	S_{mix} (% v/v)	Water (% v/v)	Pluronic F127 (% w/v)	Precipitation	% transmittance[*]	Globule size[*] (nm)	% drug entrapment[*]
ME 6	30	60	10	0.5	Yes	92.67 ± 0.43	121.6 ± 0.29	88.78 ± 0.34
ME 7	30	60	10	1	Yes	96.32 ± 0.12	109.5 ± 0.98	95.34 ± 0.65
ME 8	30	60	10	1.5	No	99.45 ± 0.54	96.71 ± 0.11	> 99%

[*]Mean ± SD; n = 3

Table 5: Physicochemical parameters of optimized batch

Parameters	Optimized batch (ME 8)
Viscosity[*] (mPas)	62.2 ± 0.98
Centrifugation	No phase separation
pH*	6.53 ± 0.21
Dilutability	No phase separation
Dye solubility test	w/o

[*]Mean ± SD; n = 3

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 96.71	Peak 1:	139.5	94.6	68.62
PdI: 0.370	Peak 2:	22.55	5.4	5.924
Intercept: 0.922	Peak 3:	0.000	0.0	0.000
Result quality: Good				

Fig. 6: Globule size analysis of bosentan-ME 8

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -15.2	Peak 1: -15.2	100.0		8.77
Zeta Deviation (mV): 8.77	Peak 2: 0.00	0.0		0.00
Conductivity (mS/cm): 0.0410	Peak 3: 0.00	0.0		0.00
Result quality: Good				

Fig. 7: Zeta potential of bosentan-ME 8

range. The surface charge on the particle measures the momentum of globules within the electric field. Optimize batch shows zeta potential -15.2 mv, which was depicted in Fig. 7. The negativity of zeta potential can govern stability. More the negative value increases stability. Zeta potential is beneficial for the measurement of physical stability of colloidal dispersions. It can be determined by the association of the globules in an electric field. Almost all aqueous colloids are electronegative, through the overall range of zeta potential is -14 to -30 millivolts.

Centrifugation

There was no phase separation/ precipitation observed in ME 8 after centrifugation at 3,000 rpm for 2 hours. It was confirmed that the system is ME. The result is shown in Table 5.

pH Measurement

The pH of optimized ME was found to be 6.53 ± 0.21, which is suitable for oral administration. The result is shown in Table 5.

Viscosity Determination

Viscosity of optimized ME was determined by digital viscometer LMDV-60. The viscosity was observed 62.2 ± 0.98 at 30 rpm using spindle SPL 1. The result is shown in Table 5.

solubilization of drug in formulation due to Pluronic F127, as there was a synergistic effect of precipitate inhibitor with surfactant and co-surfactant observed. Pluronic F 127 provides a more hydrophobic microenvironment, which was a key factor for inhibiting the drug's precipitation from formulation, allowing the drug to be held more tightly within the microstructure formed by surfactant. Globule size in the range of 96.71 ± 0.11 to 121.6 ± 0.29 nm and % transmittance was found in the range of 92.67 ± 0.43 to 99.45 ± 0.54%. The result is shown in Table 4.

Globule Size and Zeta Potential

The report of the mean globule size of the optimized batch shown in Fig. 6, which represents 96.71 nm with a low polydispersibility index, indicates globules distributed in the whole system in the narrow range. The obtained globule size was < 100 nm, which confirms ME

Dilutability and Dye Solubility Test

After diluting the optimized batch with distilled water in ratio 1:10 and 1:100, there was no sign of phase separation. From the dye solubility test, it was confirmed that ME was w/o type. The result is shown in Table 5.

Characterization of Optimized Batch of Bosentan-ME

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR investigation of pure drug and the optimized batch was compared, and the spectra of bosentan and the optimized product are shown in Table 6. FTIR spectra of bosentan and optimized product were depicted in Fig. 8. Results indicate that there was no interaction between bosentan and other excipients in the formulation. The FTIR spectra of drug and ME shows that bosentan peaks have appeared in ME, and position of pure drug peaks was not altered after the successful loading of bosentan in ME, which indicates stability of bosentan during ME process.

Transmission Electron Microscopy (TEM)

TEM image of the formulated system of bosentan in batch ME 8 shown in Fig. 9. The spherical droplet size of ME was confirmed, and drug molecules were properly incorporated into the system was confirmed by the TEM image. The result further revealed the presence of an isotropic dispersion of spherical droplets leading to the assumption of inverse micelles because of the proportions of the constituents.

Characterization of SME of Bosentan

After optimization, ME's major challenge was to convert it into a solid dosage form by adsorbing it on to the adsorbent. Here, aluminum hydroxide, magnesium hydroxide, bentonite, and Aeroperl 300 were used to adsorb ME. Their concentration was revealed in Table 7. Their physicochemical properties characterized the prepared solid ME. The data obtained were described in Table 8, and from that, it was concluded that SME 3 shows the angle of repose 30.23 ± 0.9°, which indicates good flow
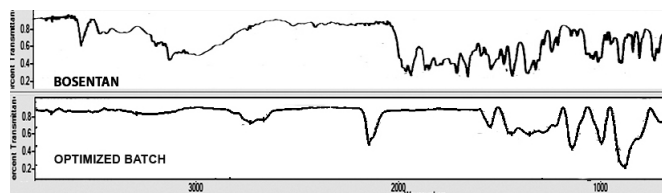

Fig. 8: FTIR spectra of bosentan and optimized batch

Table 6: Comparison of FTIR spectra of bosentan and optimize batch

S. No.	Functional group	Bosentan (cm^{-1})	Optimize batch (cm^{-1})
1	-OH	3,650	3,490.53
2	S=O	1,251	1,222.65
3	N-H	1,577	1,573.63

property. Carr's index and Hausner's ratio was established at 17.84 ± 0.08% and 1.21 ± 0.05, respectively.

Furthermore, all other absorbents except Aeroperl 300 utilized higher amounts to absorb ME, making a large quantity of powder for final formulation. The high specific surface area of 300 m^2/g, coupled with the mesopore volume of approximately 1.6 mL/g, means that Aeroperl 300 is a versatile and highly absorptive carrier that may be used to incorporate the liquids into solid pharmaceutical dosage forms. It may also assist in increasing the bioavailability of poorly soluble drugs.

In vitro Drug Release from ME, Pure Drug, and SME

As drug release is shown in Fig. 10, after 7 hours, pure drug showed 27.63 ± 0.23% CDR, liquid ME shows 78.87 ± 0.17%

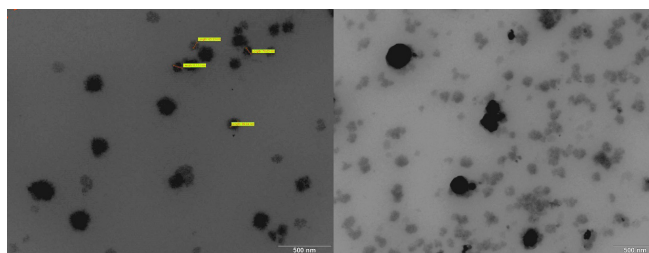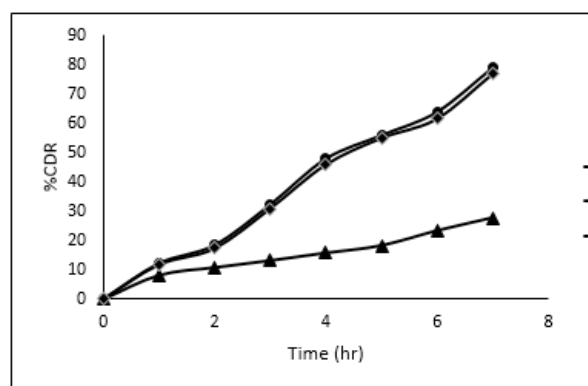**Fig. 9:** TEM image of bosentan loaded ME (ME 8)

Fig. 10: % CDR of pure drug dispersion, ME 8, and SME

Table 7: Composition of SME using adsorbent

Different adsorbent	Formulation code	Concentration in % w/w
Aeroperl 300	"SME 1"	1:0.2
	"SME 2"	1:0.25
	"SME 3"	1:0.5
Aluminum hydroxide	"SME 4"	1:1
	"SME 5"	1:2
	"SME 6"	1:4
Magnesium hydroxide	"SME 7"	1:3
	"SME 8"	1:5
	"SME 9"	1:7
Bentonite	"SME 10"	1:6
	"SME 11"	1:9
	"SME 12"	1:12

Table 8: Physicochemical parameters of SME

| Formulation	Angle of repose(°)*	Bulk density* (g/cm³)	Tapped density* (g/cm³)	Hausner's ratio*	Carr's index* (%)
SME 1	39.34 ± 0.6	0.433 ± 0.008	0.588 ± 0.09	1.35 ± 0.03	26.36 ± 0.05
SME 2	36.21 ± 0.5	0.45 ± 0.002	0.59 ± 0.02	1.31 ± 0.08	23.72 ± 0.06
SME 3	30.23 ± 0.9	0.488 ± 0.003	0.594 ± 0.04	1.21 ± 0.05	17.84 ± 0.08
SME 4	36.1 ± 0.5	0.442 ± 0.005	0.576 ± 0.03	1.3 ± 0.07	23.26 ± 0.05
SME 5	34.78 ± 0.8	0.462 ± 0.001	0.596 ± 0.01	1.29 ± 0.01	22.48 ± 0.09
SME 6	33.43 ± 0.7	0.478 ± 0.008	0.612 ± 0.06	1.28 ± 0.09	21.89 ± 0.01
SME 7	38.45 ± 0.2	0.496 ± 0.002	0.623 ± 0.02	1.25 ± 0.02	20.38 ± 0.05
SME 8	36.32 ± 0.4	0.486 ± 0.009	0.619 ± 0.09	1.27 ± 0.05	21.48 ± 0.02
SME 9	34.43 ± 0.6	0.479 ± 0.007	0.593 ± 0.03	1.23 ± 0.03	19.22 ± 0.07
SME 10	39.98 ± 0.9	0.412 ± 0.004	0.591 ± 0.06	1.43 ± 0.07	30.28 ± 0.03
SME 11	35.67 ± 0.1	0.423 ± 0.006	0.587 ± 0.04	1.38 ± 0.03	27.93 ± 0.08
SME 12	34.45 ± 0.9	0.449 ± 0.008	0.606 ± 0.08	1.34 ± 0.09	25.9 ± 0.05

*Mean ± SD; n = 3

Table 9: Pharmacokinetic parameters

Formulation	Subjects	T_{max} (hr)	C_{max} (µg/mL)	AUC_{0-t} (µg.hr/mL)	$AUC_{0-\infty}$ (µg.hr/mL)	K_{el} (hr⁻¹)	$T_{1/2}$ (hr)
Conventional	6	1.5	5.234	6.543	10.88	0.156	4.44
ME	6	1	6.421	10.231	12.56	0.105	6.6

Table 10: Stability data of SME of bosentan

Formulation	SME			
Periods (days)	0	15	30	60
Physical appearance	No change	No change	No change	No change
Angle of repose (°)	30.23 ± 0.9	30.29 ± 0.4	30.34 ± 0.7	30.42 ± 0.7
Bulk density (g/cm³)	0.488 ± 0.003	0.49 ± 0.002	0.489 ± 0.009	0.493 ± 0.005
Tapped density (g/cm³)	0.594 ± 0.04	0.596 ± 0.05	0.59 ± 0.05	0.594 ± 0.05
Carr's index (%)	17.84 ± 0.08	17.78 ± 0.07	17.11 ± 0.08	16.69 ± 0.08
Hausner's ratio	1.21 ± 0.04	1.214 ± 0.05	1.201 ± 0.06	1.204 ± 0.08
% drug content (%)	99.34 ± 0.65	99.24 ± 0.56	99.2 ± 0.34	98.97 ± 0.98

*indicate for all parameters means Angle of repose, Bulk Density, Tapped Density, Carr's index, Hausner's ratio, % Drug content
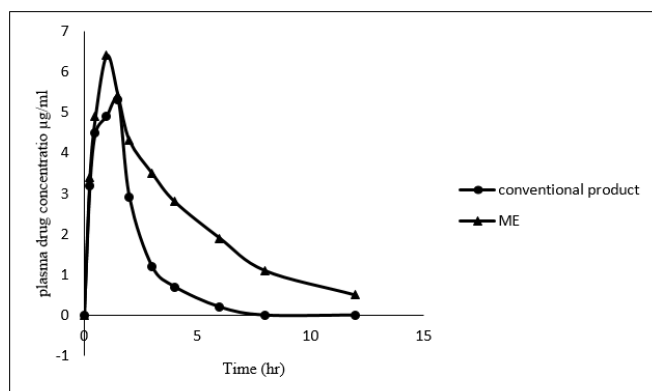
Fig. 11: Comparative plasma profile of conventional product and optimized ME formulation

CDR, and solid ME showed 76.83 ± 0.29% CDR. From the data, it was determined that ME shows better drug release compared to pure drug, which indicates there was an improvement in solubility.

Pharmacokinetic Assessment from In vivo Study

Pharmacokinetics data are shown in Table 9, which was calculated by Kinetika 5.0 software. Fig. 11 shows the comparison of plasma drug concentration between a conventional product and ME formulation. In the case of ME formulation, mean peak plasma concentration C_{max} 6.421 µg/mL achieved within 1-hour, while in conventional product C_{max} was achieved 5.234 µg/mL within 1.5 hours. The maximum concentration of ME was higher than the conventional formulation. On the other hand, decreasing in T_{max} clearly shows that the drug rapidly absorbed. This was due to the nanosize droplet of ME having large surface area. This was a direct impact on mucosal contact with intestine as a result of increased absorption. AUC_{0-t} for the conventional formulation was found to be 6.543 µg.hr/mL, which was gradually increased up to 10.231 µg.hr/mL in the case of ME. The elimination rate constant for conventional 0.156-hour⁻¹ decreases up to 0.105-hour⁻¹ for ME, which shows drugs

removed from the body at a slower rate. The half-life of ME and conventional was 6.6 and 4.44 hours, respectively, which was different from each other, shows significant importance. Thus, higher the half-life, another sign of *in vivo* presentation of ME.

Stability Study

A stability study was performed at 40 ± 2°C/ 75 ± 5% RH up to 60 days. There were no significant changes in the angle of repose, appearance, Hausner's ratio, Carr's index, bulk density, tapped density, and % drug content. The result is shown in Table 10.

Conclusion

Liquid ME was prepared by optimizing the S_{mix} ratio using ternary phase diagram. ME prepared by S_{mix} ratio (1:1) and five different formulations from the zone was selected. The main problem arose due to the precipitation of drug from the formulation, which was solved by adding precipitate inhibitor Pluronic F 127 because of its synergistic effect as solubilization with oil. Formulation having oil Capmul MCM C8 (30%), S_{mix} (60%), and Pluronic F 127 (1.5%) was optimized, having particle size 96.71 ± 0.11 nm and zeta potential -15.2 mv. Liquid ME was then adsorbed on solid carrier Aeroperl 300 and characterized for physical properties. The drug permeation study of *in vitro* liquid ME and solid microemulsion showed 78.87 ± 0.17% and 76.83 ± 0.29, respectively, significantly improved than pure drug dispersion, which only shows 27.63 ± 0.23%. The *in vivo* data of T_{max} and C_{max} confirmed that absorption of ME increased due to increasing in solubility compared to the conventional formulation. SME can be a promising method for solubility improvement.

Acknowledgment

Abbreviations

PAH: Pulmonary artery hypertension; ERA: Endothelin receptor antagonists; AUC: Area under curve; HPLC: High-performance liquid chromatography; S_{mix}: Surfactant co-surfactant mixture; USP: United States pharmacopeia; UV-spectrophotometer: Ultraviolet-spectrophotometer; ME: Microemulsion; SME: Solid microemulsion; TEM: Transmission electron microscopy; FTIR: Fourier transform infra-red spectroscopy; CDR: Cumulative drug release.

References

1. Galie N, Torbicki A, Barst R, Dartevelle P, Haworth S, Higenbottam T, *et al*. Guidelines on diagnosis and treatment of pulmonary arterial hypertension: The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. European heart journal. 2004 Dec 1;25(24): 2243-2278.
2. Galie N, Beghetti M, Gatzoulis MA, Granton J, Berger RM, Lauer A, *et al*. Bosentan therapy in patients with Eisenmenger's syndrome: a multicenter, double-blind, randomized, placebocontrolled study. Circulation, 2006;114(1): 48–54.
3. Apostolopoulou SC, Manginas A, Cokkinos DV, Rammos S. Effect of the oral endothelin antagonist bosentan on the clinical, exercise, and haemodynamic status of patients with pulmonary arterial hypertension related to congenital heart disease. Heart, 2004;91(11): 1447–1452.
4. Dingemanse J, Van Giersbergen PL. Clinical pharmacology of bosentan, a dual endothelin receptor antagonist. Clinical pharmacokinetics, 2004;43(15): 1089-1115.
5. Price LC, Howard LS. Endothelin receptor antagonists for pulmonary arterial hypertension: rationale and place in therapy. Am J Cardiovasc Drugs, 2008;8(3): 171-185.
6. Hoar TP, Schulman JH. Transparent water-in-oil dispersions: the oleopathic hydro-micelle. Nature, 1943;152(3847): 102-103.
7. Shinoda K, Lindman B. Organized surfactant systems: microemulsions, Langmuir. 1947;3(2): 35-149.
8. Varshney HM, Chatterjee A. Solubility enhancement of poorly hydrophilic drugs by using different newer techniques: A Review. Int J Therap App, 2012;6(8):13.
9. Syed HK, Peh KK. Identification of phases of various oil, surfactant/ co-surfactants and water system by ternary phase diagram. Acta Pol Pharm, 2014;71(2): 301-309.
10. Moghimipour E, Salimi A, Karami M, Isazadeh S. Preparation and characterization of dexamethasone microemulsion based on pseudoternary phase diagram. Jundishapur J Nat Pharm Prod, 2013;8(3): 105.
11. Dai WG, Dong LC, Creasey AA. Inhibiting the precipitation of poorly water-soluble drugs from Labrasol formulations. Pharma Tech, 2011;35(6): 50-54.
12. Indiran S, Sangeeta V, Rajendra K, John H, Ramya K. Microroemulsion as Solid dosage forms for oral administration, 2009;European patent. EP 2 127 642 A3.
13. Tomsic M, Podlogar F, Gasperlin M, Bester-Rogac M, Jamnik A. Water–Tween 40®/Imwitor 308®–isopropyl myristate microemulsions as delivery systems for ketoprofen: Small-angle X-ray scattering study. Int J Pharma, 2006;327(1-2): 170-177.
14. Alany RG, Tucker IG, Davies nm, Rades T. Characterizing colloidal structures of pseudoternary phase diagrams formed by oil/water/ amphiphile systems. Drug Dev Ind Pharm, 2001;27(1): 31-38.
15. Dixit GR, Mathur VB. Formulation and characterization of solid microemulsion of darunavir for enhanced solubility and dissolution. Int J Pharma Sci Res, 2015;6(9): 3990-3999.