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BOSENTAN -LOADED NANOEMULSION: A NOVEL TRANSDERMAL FORMULATION AND THEIR EVALUATION

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Abstract:

The research's main objective was to improve bosentan solubility by preparing nanoemulsion (NE) for pulmonary artery hypertension therapy. Oleic acid was selected as oil, Tween 20 as a surfactant, and PEG600 as a co-surfactant. From the pseudo ternary phase diagram ratio of Smix (1:1) selected. From the ternary diagram's NE area, different batches were prepared. The optimized formulation NE F3 contains oil (5% w/w), Smix (50%w/w), and water (44.9% w/w). The prepared was evaluated for globule size 141 nm, 99% drug content. The physicochemical parameter of NE F3 was performed, and to enhance the stability of NE, it is converted into gel, by using Carbopol 934 and Xanthan gum. The in vitro drug release investigation after 8 hours % cumulative drug release (CDR) of NE gel was 88.3 ± 0.18%, and aqueous drug emulsion (F3) shows 40.8 ± 0.13%., which indicates that NE gel revealed better drug release than NE. There was increase in solubility compare to other formulations of drug.

Keywords: *Nanoemulsion, transdermal gel, pseudo ternary phase diagram, in vitro drug release*

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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. NE 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, NE shows high drug loading, cost effectiveness, and stable formulation.[6,7]

MATERIALS AND METHODS:

Materials:

Bosentan was purchased from Yarrow chemicals India. Various oils, like oleic acid,olive oil obtained as a sample from S.D. Fine Chemicals Mumbai. Various surfactants, like Tween 20, Tween80, Span 80, and Span 20 supplied from S.D. Fine Chemicals Mumbai. Various co-surfactants, like PEG 600 and polyethylene glycol, were supplied from S.D. Fine Chemicals Mumbai other excipients like Xanthan gum triethlonamine also supplied from S. D. Fine chemicals Mumbai. Cosurfactants like PEG400 and PEG 200 is supplied from LOBA CHEMIE Laboratory Mumbai.

Methods:

study of drug in various oil:

For the selection of oil higher amount of bosentan dissolved in various oil like oleic acid, olive oil 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 0.1 N Hcl and analyzed spectrophoto-metrically at 217 nm.

Screening of surfactants and co-surfactants:

Solubility of the drug in surfactant (Tween 20, Tween 80, Span 80, and Span 20) and co-surfactant (PEG 600 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 0.1N Hcl 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 NE area can be developed. After selecting suitable components, the ternary phase diagram was developed to get the NE 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 NE 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., Smix (1:1, 1:2 and 2:1). To get a wide range of NE region, oil and Smix 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 NE:

From the ternary phase diagram, the ratio of Smix optimized. By trial and error method, various combinations of oil and Smix were selected for the preparation of NE. A fixed amount of bosentan was dissolved in oil, then added Smix, which was then titrated with water to form a clear NE. Preparation of Nano emulsion:

As Nano emulsion is a non-equilibrium system of structured liquid, the process of fabrication involves a

high amount of surfactants and external energy or both. Nano emulsion formulation can be possible by both high energy and low energy methods depending on the nature and concentration of components.

High Energy Methods:
As droplet size of Nano emulsion typically ranges from 5- 500nm, the achievement of this size involves high mechanical energy. The input of high energy for fabrication can be achieved by various methods such as high- pressure homogenizers, ultrasound generator, microfluidizer and high shear stirring method. The most significant advantage of a high energy mediated Nano emulsion formulation is the use of low concentration of emulsifiers. From the toxicity point of view, it is a major advantage.

High-pressure Homogenization:
Numerous forces such as hydraulic shear, severe turbulence, and cavitation, are frequently utilized for the development of Nano emulsions. The surfactants and co-surfactants that are passed through a small orifice of a piston homogenizer under high pressure (500-5000 psi) to generate Nano emulsions. The problem of coalescence that would occur can be solved by incorporating excess surfactants into the mixture. High-pressure homogenization is a highly effective method and a cost-effective technology that can be used on both a small and a large scale to produce Nano emulsions of extremely low particle size (up to 1 nm). The droplet sizes varies according to homogenization cycles and dispersed and continuous phase viscosities.

Micro fluidization:
This approach uses a microfluidizer device, which utilizes a high-pressure positive displacement pump (500-20,000 psi) to force the product through an interaction chamber with stainless steel microchannels on the contact area, resulting in the creation of very small sub- micron particles. The mixture is circulated through the microfluidizer till it reaches the desired particle size. The final product is filtered to separate the smaller droplets from the bigger ones and produce a homogeneous nanoemulsion.

High-speed homogenization (rotor-stator homogenizer):
High-speed homogenizers are commonly used in industry for emulsification, dispersion, and comminution. They are simple to mount in existing vessels and tanks, and they are inexpensive to buy. Rotor-stator processes are often the emulsification method of preference in many manufacturing industries. Many studies prove that it is possible to produce nanoscale droplets through using rotor-stator processes. However, this necessitates the precise selection of method and formulation parameters.

Ultrasonication:
In this method, the coarse emulsion is converted into desired nanosized emulsion droplets with the help of a sonicator probe. The sonicator probe creates high sonication sound waves of more than 20 KHz. The high intensity of sound waves breaks the coarse emulsion into fine droplets of nanosized (5-500nm). Various kinds of probes with various dimensions are available for the size reduction up to desirable limits. Along with the kinds of the probe, the input power and time for sonication decide the size of the droplet.

Low Energy Methods:
Low energy methods of Nano emulsion fabrications have more or less similar preference as high energy methods and include various methods such as spontaneous emulsification, phase inversion methods, emulsion inversion points etc. However, in comparison to high energy methods of Nano emulsion preparation. Higher amount of surfactants is used, which provides smaller globules of low and uniform polydispersity index (PDI). These low-energy methods make use of the phase transitions that take place during the emulsification process as a result of a change in the spontaneous curvature of the surfactant.

Spontaneous Emulsification Method:
It is one of the most feasible methods of Nano emulsion preparation on lab and industrial scale and involves two liquid phases, one aqueous phase and another is an organic phase. The aqueous phase consists of a hydrophilic surfactant of various categories such as Tween, span etc. and the organic or oil phase consists of components such as Capriole 90 or any similar material such as Acetone, Ethyl methyl ketone etc. in which drug is pre-solubilized. In this process, the spontaneous formation of Nano emulsion is achieved when the organic phase is added slowly to the aqueous phase and followed by evaporation of the organic phase.

Phase inversion temperature technique (PIT):
In phase inversion temperature (PIT) method, the change in geometry of the interface of the surfactant (mainly non-ionic in nature) is achieved by changing the temperature of the formulation and the composition is kept constant. With a gradual increase in temperature, the coarse emulsion gets heated up and causes the surfactant to get solubilized in oil phase resulting in the transformation of o/w emulsion to w/o emulsion.

Phase Inversion composition (PIC):
In phase inversion composition (PIC) method the temperature is kept constant and change in the composition is allowed for the inversion process. Most preferably pseudo ternary phase diagram method is used for the formulation of Nano emulsion using phase inversion composition method. The major disadvantage of this method over higher energy required methods is that the surfactant is used in higher amount in it[12,13]

Preparation of Nanoemulgel:
The gel base is produced by dissolving the polymer in purified water and continually stirring it with a mechanical stirrer. Following the preparation of the Nano emulsion and the gelling agent, the two are continuously stirred until a nanoemulgel is formed. Water in oil (w/o) or oil in water (o/w) Nano emulsion is turned into thick and semisolid nanoemulgel with the aid of different polymeric gelling agent. Formulation of Bosentan Nanoemulsions:
After the nanoemulsion regions in the phase diagrams were identified from the phase studies, 1:1 ratio of Smix was finalized for formulation of nanoemulsions. Concentrations of the components were selected on the basis of pseudo ternary phase diagram by varying concentration of oil and smix at 3 levels and the total of 9 formulations were prepared and denoted as F1 to F9.[11]

Preparation of Bosentan Nanoemulsions:
NEs were prepared by the ultrasonic emulsification method by using probe sonicator (PCI, Mumbai) Ultrasonic emulsification is known to be a reliable method for the production of long-term stable nanoemulsions that can reduce the droplet size of primary emulsion to size less than 200 nm.

This involves either of two phenomena (1) waves generated by acoustic field travel through the liquid and produce micro turbulences and an interfacial movement occurs. Because of this, the boundary phase becomes unstable and the dispersed phase. eventually breaks into droplets in the continuous phase. (2) The application of ultrasonic waves of low- frequency and high power generates cavities that lead to the formation of microbubbles or voids in the medium.
These extreme forces are responsible for the primary droplets of dispersed phase to break down into nano-sized droplets and mix them homogeneously into the continuous phase.

The drug was dissolved in selected oil and then the Smix was added, the aqueous phase was then added to the oil phase with continuous stirring to produce a primary emulsion, the most common method for preparing highly concentrated emulsions. The primary emulsion was then sonicated to get a nanoemulsion following a cycle of 20 min.[12]

Compositions of nanoemulsion formulation:

Table no 1: composition of nanoemulsion

Sr. No.	Formulation	Oil(Oleic acid) (% w/w)	Smix (1:1) (Tween 20: Polyethylene glycol) (% w/w)	Water(%w/w)	Drug (%w/w)
1.	F1	10	30	59.9	0.1
2.	F2	5	40	54.9	0.1
3.	F3	5	50	44.9	0.1
4.	F4	7.5	40	51.5	0.1
5.	F5	7.5	60	32.4	0.1
6.	F6	10	40	49.9	0.1
7.	F7	5	50	44.5	0.1
8.	F8	10	50	39.9	0.1
9.	F9	5	60	34.9	0.1

Formulation and Evaluation of Nanoemulsion Based Gel:
In order to improve the patient compliance nanoemulsion can be converted in the form of transdermal gels by employing a suitable polymer that is capable of modifying rheological behavior of nanoemulsions. This nanoemulsion based gel amalgamate advantages of nanoemulsions and gel which helped in improving the ease of handling and application. Several gelling agents have already been studied for their potential to form nanoemulsion. However, it was essential to ensure that the polymer does not alter the desirable features of nanoemulsions.

Screening of Polymer used in the Preparation of Nanoemulsion Gel:
Various gelling agents namely, HPMC K4, Carbopol 934 and xanthan gum were used to prepare transdermal gel of nanoemulsion. The different concentrations (0.2%, s0.5%, 1%, 1.5% and 2%) of gelling agents were used to determine the concentration at which acceptable transdermal gel was formed as shown in Table below, Carbopol 934 and xanthan gum has been reported for use in topical drug delivery systems. Briefly, gelling agents were directly added to the prepared nanoemulsion slowly with continuous stirring with the help of the overhead stirrer. In case of Carbopol 934, the dispersion was neutralized by using 1 % (w/w) Triethanolamine to obtain gel

Evaluation:
Appearance:
All batches were evaluated visually for clarity and any sign of precipitation. Alternatively, under the white and black background, all NE formulations were examined by the naked eye to observe any turbidity sign. The test was performed as per United States pharmacopeia (USP).[10]

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.[11]

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 NE. NE'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 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-NE was measured by photon correlation spectroscopy (Zetasizer Nano ZS, Horiba Instruments). The sample was diluted with distilled water and then analyzed.

Zeta Potential:
To observe the physical stability of NE, 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, NE was diluted 10 times with distilled water and then analyzed by (Zetasizer Nano ZS, HORIBA Instruments).

Drug Content:
NE equivalent to 10 mg of bosentan was dissolved in 0.1NHcl (100 mL). The mixture was thoroughly mixed to dissolve the drug in Hcl, and absorbance measured by UV-spectrophotometer at 217 nm.

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

In Vitro Diffusion Study:
Most of the work on in vitro release from semisolids uses vertical Franz-type cells. The cell body consists of a glass receptor chamber having capacity 25 ml in volume (modified cells exist with slightly different

receptor volumes) and a glass sampling port. The membrane is placed horizontally over the receptor chamber, the cell cap is applied over that, and the components are held together with rubber bands. The test formulation can then be applied to the surface of the membrane through the top of the cell cap, which is open to the atmosphere unless sealed. A micro magnetic stirrer is placed at the bottom of the receptor chamber containing release media.

The in vitro drug release profile of optimized nanoemulsion based gel as well as nanoemulsion formulation were determined in phosphate buffer pH 7.4, using dialysis membrane-60 (Hi- media, Mumbai) at 37 °C under magnetic stirring at 100 RPM. The diffusion was carried out for 8 hours and 1ml sample was pipetted out at an interval of 1 hour.

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 NE like its physicochemical properties, appearance, and drug content.

RESULT AND DISCUSSION:

Solubility of Bosentan in various Oils:

The solubility of the drug in various oil depicted in Fig.1, like oleic acid, Labrafac PG, Captex 355, Capmul MCM C8, and Captex 200P. In this study, Capmul MCM C8 shows the highest solubility 18.94 mg/mL, which was selected for further investigation.

Screening of Surfactants and Co-Surfactants:

Solubility of the drug in surfactant (Tween 20, Tween 80, Span 80 and Span 20) and co-surfactant (PEG400, PEG600, and PG) were depicted in Fig1. From the different surfactant and co-surfactants, Tween 20 and PEG600 were selected as they show the highest solubility 32.92 and 17.57 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.

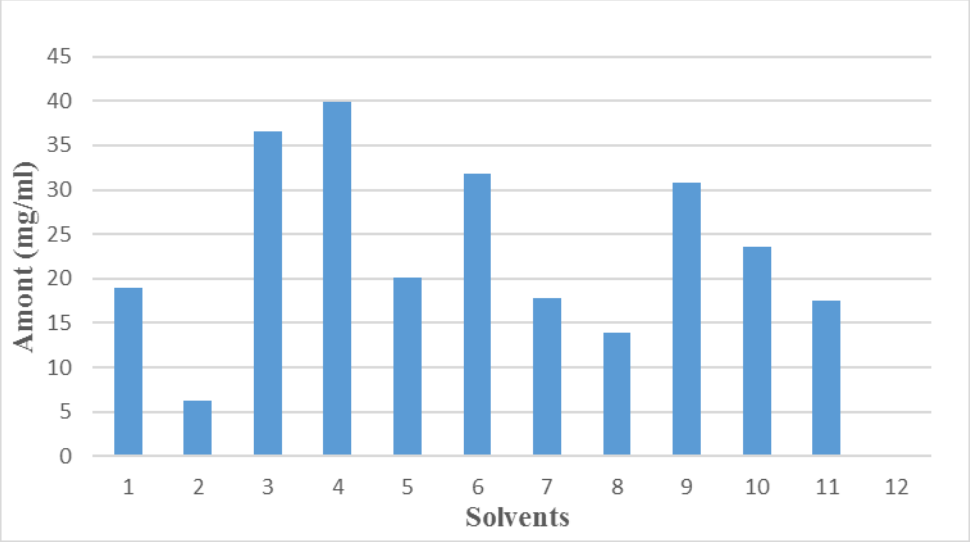

Figure1: Solubility of Bosentan in different Solvent

Optimization and Characterization of Bosentan Nanoemulsion:

Pseudo-ternary phase diagrams:

Pseudo ternary phase diagrams were constructed separately for each surfactant to the co surfactant ratio and were given in figure below. As shown in figure 1:1 and 1:2 ratio had larger areas than that of 2:1 From 1:1 and 1:2 ratio, large nanoemulsion area was observed for the ratio 1:1 (w/w) and the same was used to optimize the final formulation compositions.Also, concentration of Tween 20 increases water solubility and decreases solubility, therefore 1:1 is selected for further studies.More Concentration of tween 20, oleic acid and PEG decreases drug solubility and main objective of formulation is to increase solubility and

bioavailability of drug for its desired effect therefore 1:1 ratio is more suitable for further procedure.

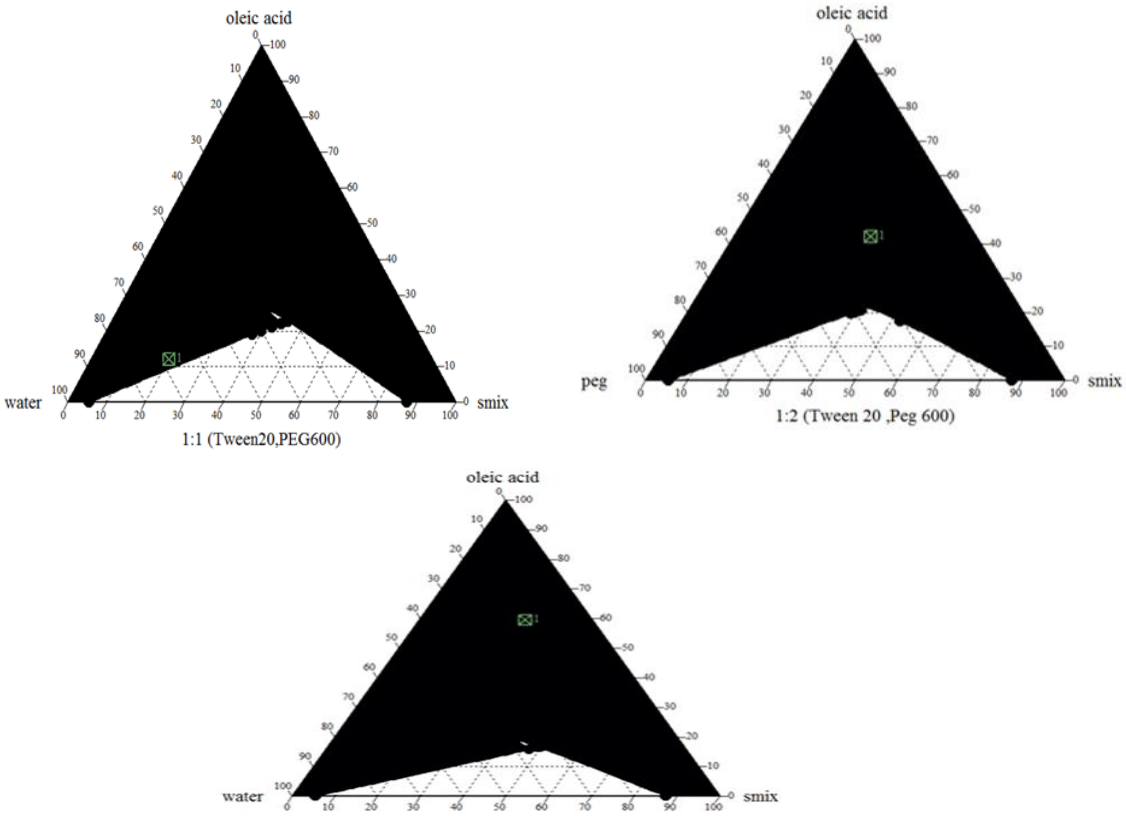

Figure 2 :Pseudo ternary phase diagrams of Oleic acid and Smix (Tween 20: Polyethylene glycol)Ratios; a) 1:1, b) 1:2 and c)2:1

Formulation and Preparation of Bosentan Nanoemulsions: [12,13]
Concentrations of the components were selected on the basis of optimized pseudo ternary phase diagram and the formulations were prepared as per procedure with addition of drug

Characterization of Bosentan Nanoemulsions:
Macroscopic appearance:
Before dilution with water, nanoemulsions were qualitatively evaluated for appearance, colour and consistency. Nanoemulsions were found to be clear, transparent and homogeneous liquids. No particulate matter of drug precipitation was observed in these monophasic liquids.
Refractive Index:
Nanoemulsions were observed for refractive index using Abbes refractometer. The refractive index values of the formulations are as indicated in the table 8.5. Formulations showed refractive index close to the water indicating the clarity.
pH:
The pH values of the nanoemulsion formulations having concentration of one gram of nanoemulsion in 50 ml of water are shown in the table 8.5. As shown in the table, the pH values of all formulations were found to be compatible for topical formulation.
Drug Content:
The drug content of Bosentan in the nanoemulsion was determined in triplicate by UV spectroscopy at λmax of 217nm. The drug content in the nanoemulsion is as shown in the table 8.5, and was well within limits. There was no degradation of drug in the nanoemulsion as only single peak of Bosentan was observed.
Viscosity:
The viscosity of nanoemulsion formulations were measured using Brookfield viscometer and the values are reported.

Tanle no 2: Characterization parameters of Bosentan nanoemulsions

Sr no.	Formulation	Refractive index (±SD)	pH	Drug content	Viscosity (mpa. s)
1.	F1	1.38±0.018	6.36±0.12	84.76±0.1	536.4
2.	F2	1.55±0.018	6.42±0.12	89.95±0.2	525
3.	F3	1.35±0.018	6.33±0.12	99.53±0.17	501.4
4.	F4	1.58±0.018	6.20±0.12	98.51±0.1	551
5.	F5	1.59±0.018	6.46±0.12	95.85±0.16	524
6.	F6	1.57±0.018	6.45±0.12	98.09±0.03	644
7.	F7	1.59±0.018	6.36±0.12	96.20±0.1	759.3
8.	F8	1.54±0.018	6.50±0.12	99.89±0.11	837.9
9.	F9	1.55±0.018	6.23±0.12	98.62±0.05	435.9

Particle size analysis (Globule size determination): Mean globule size was ranging from 141 to 230 nm and all system showed single peak in size distribution. The results of particle size analysis of the different formulations with Bosentan are presented in table below.

The particle size of formulation F1, F2, F3 and F4 were found in the range of 20-200nm, which is generally considered to be the particle size of nanoemulsion. While in case of F5, F6, F7, F8 and F9 particle size is larger than 200nm. It may be due to lower conc. of surfactant/cosurfactant but it also can be considered as nanoemulsion as some authors have demonstrated particle size range of nanoemulsion 20-500nm, also these formulations are optically clear and transparent by visual observation. The particle size of formulation F3 was smallest i.e., 141 nm as compared to other formulations. The graph of the intensity of the F3 particle size is shown in figure below. The results showed that the smaller particle size of nanoemulsions was obtained due to the higher concentration of surfactant and cosurfactant. The decrease in particle size can be attributed to the solubilization of internal phase within a larger number of surfactant micelles, which are consequently swollen to a lesser extent.

Table no 3: Particle size of Nanoemulsions

Sr. No.	Formulation	Particle Size (nm)
1.	F1	159
2.	F2	145
3.	F3	141
4.	F4	142
5.	F5	215
6.	F6	227.7
7.	F7	238
8.	F8	218.9
9.	F9	230

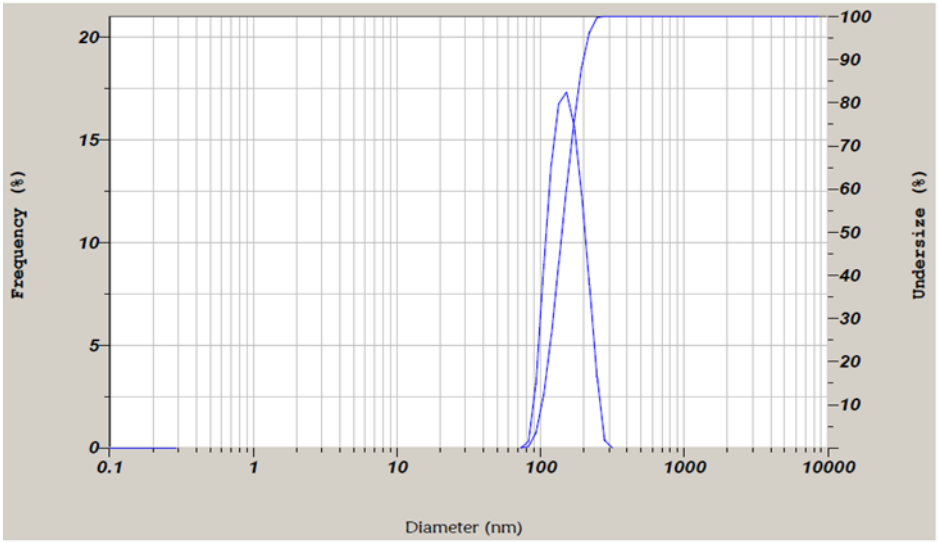

Figure3: particle size distribution graph of F3 formulation

	Peak	Size (nm)
Z-Average(nm): 154.7	Peak1	141.2
PDI: 0.293	Peak2	141

Particle size analysis of F3 formulation

Zeta Potential:

The zeta potential is an important and readily measurable indicator of the stability of nanoemulsions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. The zeta potential value and its graph is shown in the table and figure below. F3 nanoemulsion has been shown to be stable due to the presence of non-ionic surfactants that impart stability to the system by steric stabilization, considering the lower zeta potential value obtained. Adsorption of these steric stabilizers decreases the zeta potential value and produces strong repulsion between particles thereby preventing aggregation during storage.

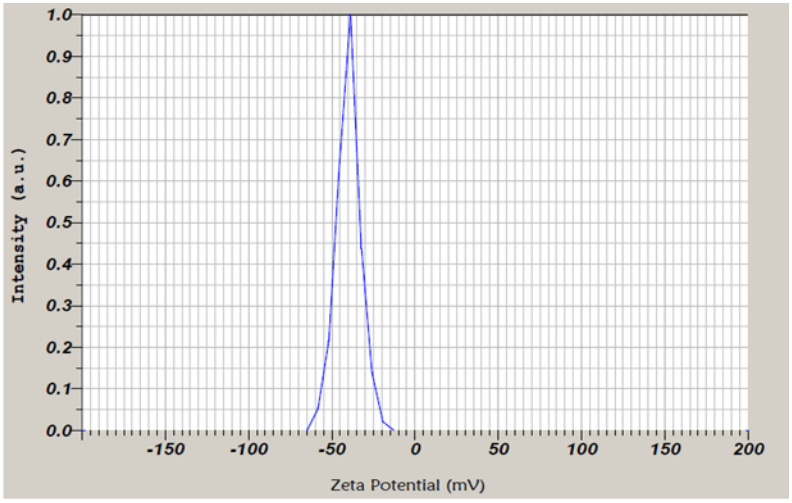

Figure4: Zeta potential distribution of F3 nanoemulsion

Table no 4: Results of zeta potential distribution of nanoemulsion

	Peak	Mean (mV)
Zeta Potential(mV): -40.1	Peak 1	-40.1
Conductivity (mS/cm): 0.065	Peak 2	-40

From particle size analysis and zeta potential, it was found that F3 nanoemulsion has lowest particle size 141 nm among all the nanoemulsion formulations and the zeta potential of the F3 formulation was found to be -40.1 mV showing that the respective formulation is stable due to steric stabilization of similarly charged particles.

Hence, it can be concluded that F3 formulation is the optimized batch and having good and satisfactory results**.**

Formulation and evaluation of Nanoemulsion Based Gel [14]

Screening of Polymer used in the Preparation of Nanoemulsion Gel

Following gelling polymer were screened for preparation of nanoemulsion based gel as shown in the table. HPMC K4 was unable to give the gel of appropriate consistency. Carbopol 934 was able to give clear transparent gel at given concentration ranges. Xanthan gum showed transparent smooth gel upto 1% concentration and above that it formed slimy masses. Therefore, Carbopol 934 (0.2%w/w) and Xanthan gum (0.2%w/w) in combination were selected to form nanoemulsion gel of optimized nanoemulsion.

Table no 5: Screening of gelling polymers

Sr. No.	Gelling Agents	Conc. Of Gelling Agent
1.	HPMC K4	0.1 – 5%
2.	Carbopol 934	0.1 – 5%
3.	Xanthan Gum	0.1– 5%

Preparation of Nanoemulsion Gel:

From above study, Carbopol 934 (0.2%w/w) and Xanthan gum (0.2%w/w) in combination were selected as gelling agent for optimized nanoemulsion formulation (F3) and prepared as per procedure.

EVALUATION OF NANOEMULSION GEL:

pH:

The pH value of prepared nanoemulsion gel was determined and found to be 7.12 which is acceptable and compatible for topical formulation.

Spreadability:

The spreadability of the prepared nanoemulsion gel was determined and found to be 5 g.cm/s indicating that the spreadability of the nanoemulsion gel was good.

Extrudability Study:

The extrudability of the prepared nanoemulsion gel was determined and found to be 1.1 g/cm2 indicating that the extrudability of the gel was good.

Drug content:

The drug content of Bosentan in the nanoemulsion was determined in triplicate by UV spectroscopy at λmax of 217 nm. The drug content of nanoemulsion gel was found to be 99%w/w. There was no degradation of drug in the nanoemulsion as only single peak of Bosentan was observed.

Sr.No.	Parameters	Observations
1.	PH	7.12±0.12
2.	Spreadability	5g.cm/s
3.	Extrudability	1.1g.cm/s
4.	Drug content	99%w/w

Table no 6: characterisation parameters of nanoemulsion gel

Rheological study:

The shear thinning effect is ascribed to orientation of the liquid crystalline domains in the direction of the shear stress vector and structural breakdown.

This results in reduced resistance to flow and hence a decrease in viscosity

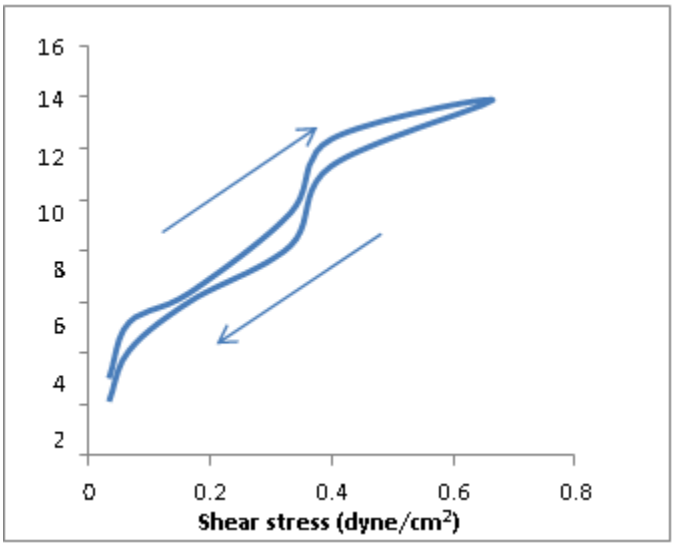

Figure 5: Rheology of nanoemulsion gel

In Vitro Diffusion Study:

In vitro release studies were carried out in using dialysis-60 membrane to assess the release of Bosentan nanoemulsion gel and marketed formulation The diffusion cell selected was Franz-diffusion diffusion cell. Saline Phosphate buffer (pH 7.4) was selected as diffusion medium on the receptor side.

The in vitro release studies were carried out for 8 hrs using Franz diffusion cells. From the in vitro release profile, it was clear that about 83% drug release was achieved in 8 hrs from the formulation containing Carbopol 934 (0.2%) and xanthan gum (0.2%) in combination as gelling agent. And about 40% of drug was released from the aqueous drug emulsion resulting that the nanoemulsion gel formulation shows better release profile than the aqueous drug emulsion.

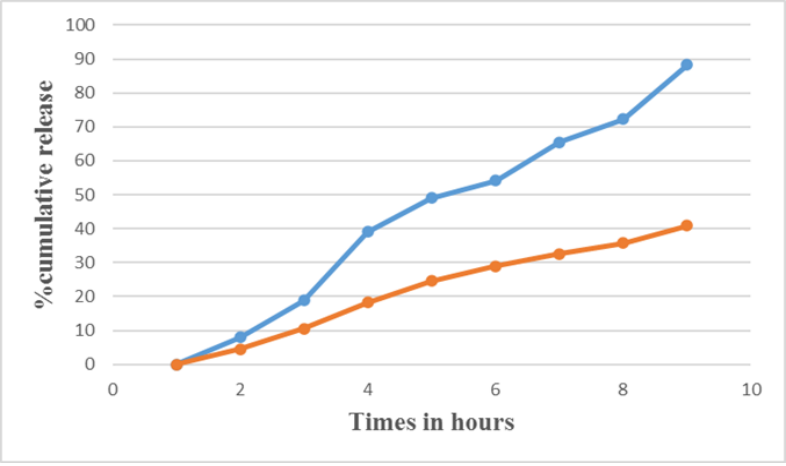

Figure 6: Cumulative release of nanoemulsion gel and aquous drug emulsion

CONCLUSION :

In this study, an attempt was made to formulate Nanoemulgel formulation of Bosentan for Transdermal delivery. Based upon the experimental findings it can be concluded that:

Solubility study revealed that the Bosentan had solubility in Oleic acid among all the oils, tween 20 and polyethylene glycol among the surfactants and co-surfactants respectively. Drug excipient compatibility study showed no shift in λmax of the overlay spectrum of drug and drug + excipient indicating no interaction between drug and excipients. Phase behavior investigations of selected excipients demonstrated a suitable approach to determine the ratio of smix and concentration range of various components used, over which they could form nanoemulsions. In view of current investigation, due to larger nanoemulsion region oleic acid- tween 20: polyethylene glycol-water system with smix ratio of 1:1 was selected for further formulation studies. Formulations of nanoemulsion containing Bosentan in the concentration 0.1%w/w were prepared and characterized for pH, globule size, drug content and zeta potential. From characterization results of nanoemulsions, F nanoemulsion was found to have lowest globule size and hence considered to be optimized. Among the polymers screened for the preparation of gel, Carbopol (0.2%w/w) and xanthan gum (0.2%w/w) in combination were found to be suitable and utilized to prepare nanoemulsion gel of optimized formulation F3 by using overhead stirrer and evaluated for various parameters as pH, spreadability, drug content and rheological studies. Rheological study revealed the pseudoplastic behavior of nanoemulsion gel. In vitro release profile was found to be better for nanoemulsion gel. Nanoemulsion gel showed stability over different storage conditions.

FUTURE SCOPE:

Bosentan nanoemulgel as transdermal delivery system to treat hypertension (PAH)can be a promising delivery system as there are limited treatment options available to treat hypertension (PAH) without first pass metabolism. So nanoemulgel as transdermal delivery could be useful to utilize lipophilic drugs in hydrophilic structure of gel which gives better treatment for hypertension from the present investigation, it can be concluded that nanoemulgel can be a safe, effective and promising formulation for the transdermal treatment of Pulmonary Arterial hypertension.

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