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(54) Title: FORMULATIONS OF BOSENTAN MONOHYDRATE LOADED SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS (SNEDDS) PREPARED WITH LONG-CHAIN MONO AND DIGLYCERIDE MIXTURES

(57) Abstract: The invention relates to the preparation, optimization, and characterization of the formulations of liquid self-nanoemul-sifying drug delivery systems (SNEDDS) containing bosentan monohydrate (BOS) using long-chain mono and diglyceride mixtures. BOS-containing liquid SNEDDS, Glyceryl monolinoleate (Maisine®) - Polyoxyl 40 hydrogenated castor oil (Cremophor® RH 40) - Caprilcaproil polyoxyl-8 glycerides (Labrasol®) and Glyceryl monooleate (Peccol®) - Polyoxyl 40 hydrogenated castor oil (Cre-mophor® RH 40) - Caprylocaproyl polyoxyl-8 glycerides (Labrasol®) from certain proportions of formed, and the systems were de-termined by water titration method.



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FORMULATIONS OF BOSENTAN MONOHYDRATE LOADED SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS (SNEDDS) PREPARED WITH LONG-CHAIN MONO AND DIGLYCERIDE MIXTURES

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TECHNICAL AREA

It is concerned with the preparation, optimization, and characterization of liquid SNEDDS formulations determined by the water titration method containing bosentan monohydrate (BOS) using long-chain mono and diglyceride mixtures.

10 **BACKGROUND OF THE INVENTION**

Today, more than 70% of newly discovered drug candidates appear to be poorly water-soluble, lipophilic, low bioavailability, high intra-, and inter-subject variability or low solubility, and high permeability Class 2 compounds according to the Biopharmaceutical Classification System (BCS). Although these BCS Class 2 compounds have very high membrane permeability throughout the intestine their solubility in gastrointestinal fluids is very limited, so their absorption is also limited. Therefore, formulation approaches used to increase the bioavailability of such drugs focus on improving the dissolution and solubility properties of the drug or overcoming the dissolution problem by developing a new alternative dosage form to the conventional dosage form. Different techniques are used (such as particle size reduction, nanonization, co-solvent use, surfactant use, functional polymer technology, controlled precipitation, inclusion complex, nanoparticle technology, nanocrystal technology, solid dispersion technique, inclusion complex, and self-emulsifying systems) to increase the solubility, dissolution rate, and thus bioavailability of water-insoluble drugs.

Lipid-based formulations are considered a well-known strategy for increasing solubility and oral bioavailability, minimizing the in vivo variability of poorly soluble compounds. Various dosage forms, including solutions, emulsions and self-emulsifying/microemulsifying /nanoemulsifying drug delivery systems (SEDDS/SMEDDS/SNEDDS) can be developed to increase the solubility and bioavailability of BCS Class 2 and BCS Class 4 drugs using the lipids.

Self-nanoemulsifying drug delivery systems form a self-oil/water nanoemulsion smaller than 100 nm under gentle agitation with the aqueous phase in the gastrointestinal tract. The systems contain natural or synthetic oils, solid or liquid surfactants, and co-surfactants, or isotropic mixtures of more hydrophilic solvents. These systems are used to improve the dissolution and absorption

of BCS Class 2 drugs. SNEDDSs not only show a remarkable increase in dissolution rate, solubility, and permeability, but also significantly reduce intra- and inter-individual variability, food effect, p-gp efflux, and the first pass through the liver depending on conditions in the gastrointestinal tract. In addition, it has advantages such as increasing stability by protecting drugs
5 from the gastrointestinal environment, increasing drug loading capacity, ease of production and scale-up and obtaining more consistent blood concentration-time profiles. SNEDDS can provide increased physical and chemical stability, taste masking, and patient compliance when used in capsules as single dosage forms. There are many drugs developed in the form of self-emulsifying systems on the market which are Cyclosporine A (Sandimmune[®]/Neoral[®]), enzalutamide
10 (Xtandi[®]), lubiprostone (Amitiza[®]), calcitriol (Rocaltrol[®]), fenofibrate (Lipofen[®]), ritonavir (Norvir[®]), and lopinavir-ritonavir (Kaletra[®]).

Bosentan monohydrate (BOS) is an orally administered non-peptide endothelin receptor antagonist specifically for the treatment of pulmonary arterial hypertension (PAH). There are 62.5 and 125 mg film-coated tablet dosage forms on the pharmaceutical market for use in the treatment
15 of PAH. For the therapeutic effect of the products to be observed in patients, it should be used at a dose of 62.5 mg twice a day for the first four weeks of treatment, and then at a dose of 125 mg twice a day. This treatment requires lifelong drug use. This treatment is quite expensive. Therefore, the economic cost of this health expenditure is quite high for both the patient and the society.

EMA's report on Tracleer[®] 62.5 mg - 125 mg dosage forms states that the increase in liver aminotransferases (AST and ALT) in long-term BOS use is dose-dependent and that ALT-AST levels should be monitored against the risk of liver damage during the treatment. This increase is typically seen in the first 26 weeks of treatment, but can also be seen in the later stages of treatment. These increases may be due, in part, to competitive inhibition of bile salts elimination
25 from hepatocytes, and although not conclusively identified, other mechanisms are thought to be associated with hepatic failure. It should be considered in the treatment that BOS will accumulate in hepatocytes and may cause possible serious liver damage or cytolysis up to an immunological mechanism. In the case of clinical signs associated with nausea, vomiting, fever, abdominal pain, jaundice, unusual lethargy or fatigue, flu-like syndrome (arthralgia, myalgia, and fever) due to liver
30 damage, treatment should be stopped, and the situation should be evaluated. In addition, the decrease in hemoglobin level during treatment was also bosentan dose-dependent.

It is a convenient strategy to overcome the solubility and bioavailability problem and dose-dependent side-effect problem of bosentan, which is a BCS Class 2 drug, with appropriate

formulation strategies, to increase its bioavailability and treatment efficacy, and to reduce the dose amount/number of side effects.

In the literature, it was aimed to increase the solubility/dissolution rate of the active substance and thus to increase its effectiveness by preparing osmotic controlled tablets containing bosentan monohydrate, solid dispersion, extended-release matrix tablets prepared using synthetic/natural polymers, mucoadhesive microsphere, extended-release pellet, microspheres prepared with biodegradable polymer mixture, controlled release system, transdermal patch, inhaled controlled release polymeric colloid, fast-dissolving nanosuspension, nanocomposite, self-microemulsifying, and self-nanoemulsifying system dosage forms.

10 There are SEDDS and SNEDDS formulation studies containing bosentan in the literature. The SEDDS formulation study was conducted by Gunnam et al. In this study, Gelucire 44/14 as oil, Cremophor EL as surfactant, and polyethylene glycol 400 (PEG 400) as co-surfactant were selected. The droplet size was 37.8 ± 6.2 nm, and polydispersity index (PDI) was 0.279. The stability study was also carried out for 15 days and 1 month. The in vitro dissolution rate study
15 was performed in 900 mL of distilled water, only a 1.8-fold increase in the cumulative amount of % dissolved drug was observed. Another SNEDDS formulation study was conducted by Panigrahi et al. In this study, Capmul MCM, Labrasol, and PEG 600 were selected as oil, surfactant, and co-surfactant, respectively. The droplet size was 62.5 nm, the PDI was found to be 0.146. The acceptable results were obtained for 6 months in the stability study. The in vitro dissolution study
20 was performed in 0.1 N HCl medium (pH 1.2) for SNEDDS. The 1.6-fold increase was observed in the % average amount of drug released compared to pure active substance alone.

The design of a self-nanoemulsifying system with long-chain mono and diglyceride mixtures has not been tried before for bosentan monohydrate. According to the SEDDS and SNEDDS studies encountered in the literature with the invention, it was thought that more optimized, robust, stable,
25 and stable self-nanoemulsifying systems could be developed with long-chain mono and diglyceride mixtures and that the developed formulation would show a higher in vitro dissolution profile in biorelevant media. For this purpose, in vitro dissolution rate studies were carried out in distilled water containing 1% SLS, which is the dissolution media recommended by the FDA, and in dissolution media that mimic fasted (FaSSIF = Fasted State Simulated Intestinal Fluid) and fed
30 (FeSSIF = Fed State Simulated Intestinal Fluid), which are biorelevant media that are thought to better reflect in vivo. It is also clear that designing the SNEDDS to increase the solubility, in vitro dissolution rate and bioavailability of bosentan monohydrate will be more advantageous than the

solid oral dosage form (Tracleer® film tablet) in the drug market, considering the advantages of use for the patient and the possible pharmacoeconomic reduction of treatment costs.

DESCRIPTION OF THE INVENTION

To increase the in vitro dissolution and bioavailability of bosentan monohydrate, which has a very low water solubility and dissolution, the liquid SNEDDS formulations were prepared with long-chain mono and diglyceride mixtures. Glyceryl monolinoleate (Maisine®) and glyceryl monooleate (Peceol®) as oil, which is long-chain mono and diglyceride mixtures, polyoxyl 40 hydrogenated castor oil (Cremophor® RH 40) as surfactant, and caprylocaproyl polyoxyl-8 glycerides (Labrasol®) as co-surfactant were used in the preparation of SNEDDS. The water titration method was chosen for the preparation of a liquid SNEDD system using long-chain mono and diglyceride mixtures.

Design Expert® Software Version 10 was used for the preparation of liquid SNEDDS containing optimum long-chain mono and diglyceride mixtures without active substance. The droplet size and polydispersity index (PDI), evaluation of self-emulsification efficiency, % transmittance, the effect of pH and liquid volume on droplet size and PDI, turbidity, thermodynamic and long-term stability, morphological characterization studies have been carried out in optimum formulations without the active substance and after loading bosentan monohydrate.

In vitro dissolution studies were performed using distilled water media containing 1% SLS, which is the in vitro dissolution media recommended by the FDA, FaSSIF, and FeSSIF, which are biorelevant media. In the literature, there is no evaluation of the in vitro dissolution for bosentan-loaded SNEDD systems using the biorelevant dissolution media, FaSSIF and FeSSIF.

As a result, bosentan monohydrate was successfully loaded into liquid SNEDDS prepared with long-chain mono and diglyceride mixtures and passed the all characterization studies successfully. Compared to the commercial product, the prepared new system provided a much higher dissolution profile in fasted and fed states media that mimicked the gastrointestinal tract.

In distilled water medium containing 1% SLS, more than 80% release of bosentan was observed within 15 minutes from the SNEDDS formulation containing BOS-loaded glyceryl monolinoleate and glyceryl monooleate and the commercial tablet, while all of the active substance was released within 30 minutes. On the other hand, in the FaSSIF media, which mimics the gastrointestinal environment much better than the SLS-containing media, the commercial tablet were released about 33% of the active substance at the end of 90 minutes, while the BOS-loaded glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations were released approximately 69%

and about 83% within 15 minutes, respectively. At the end of 90 minutes, BOS-loaded glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations released 95% and 96% of the active substance in their content, respectively. In the FeSSIF media, while the commercial tablet released approximately 11% of the active substance at the end of 90 minutes, the BOS-loaded glyceryl monooleate SNEDDS formulation released 85%, and the BOS-loaded glyceryl monolinoleate SNEDDS formulation 86% at the end of 90 minutes.

The ratio of components in liquid SNEDDS formulations prepared with long-chain mono and diglyceride mixtures is 0.9:7.2:0.8 by weight, as oil:surfactant: co-surfactant. Accordingly, the ratio of glyceryl monolinoleate, polyoxyl 40 hydrogenated castor oil, and caprylocaproyl polyoxyl-8 glycerides and the ratio of glyceryl monooleate, polyoxyl 40 hydrogenated castor oil, and caprylocaproyl polyoxyl-8 glycerides by weight are 0.9:7.2:0.8 (w/w/w).

In BOS-loaded glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations, the droplet size is below 50 nm, and the PDI is less than 0.2.

The effect of pH and liquid volume on PDI, after thermodynamic and long-term stability studies, droplet sizes are less than 50 nm, PDI values are less than 0.2

1 gram of glyceryl monolinoleate SNEDDS formulation comprises 30 mg of bosentan monohydrate.

1 gram of glyceryl monooleate SNEDDS formulation comprises 28 mg of bosentan monohydrate.

20 **Example 1**

Obtaining Liquid SNEDDS with Ternary Phase Diagram

Solubility studies of bosentan monohydrate in different oil groups have been carried out. Considering the hydrophilic-lipophilic balance values (HLB), the highest solubility glyceryl monolinoleate, polyoxyl 40 hydrogenated castor oil, caprylocaproyl polyoxyl-8 glycerides were chosen as oil, surfactant, and co-surfactant. One type of mixture was obtained by screening the surfactant and co-surfactant ratio between 9:1 to 1:9. S_{mix} (surfactant: co-surfactant ratio) was determined as 9:1. The obtained homogeneous mixtures were then mixed with oil in the ratio of 9:1 – 1:9 to create a homogeneous system. These systems were then titrated with water, and the boundaries of the ternary phase diagrams containing the nanoemulsion field were determined.

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Optimization of SNEDDS Formulation

It was investigated whether nanoemulsion was formed by diluting different ratios with 1:250 distilled water within the borders of the triangular phase diagrams. Optimum glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations were created by evaluating the determined extreme values as oil, surfactant, and co-surfactant for independent variables, and droplet size and PDI for dependent variables using the Box Behnken design Design Expert® program. The robustness of the obtained formulations was supported by characterization studies. Based on the SNEDDS formulation supported by studies, BOS- loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations were prepared. Different amounts of active substance were tested and mixed with blank formulations and vortexed until homogeneous preparations were obtained. Then it was allowed to dissolve completely in a 37°C water bath for 24 hours, and the obtained bosentan monohydrate loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations were stored at room temperature until use. In this study, 30 mg BOS was loaded into a 1 g glyceryl monolinoleate SNEDDS formulation, and 28 mg BOS was loaded into a 1 g glyceryl monooleate SNEDDS formulation.

Characterization Studies of Glyceryl Monolinoleate and Glyceryl Monooleate SNEDDS Formulations Without Active Substance and BOS-Loaded

To evaluate the self-emulsification efficiency, dispersibility, and self-emulsification time studies were carried out. In dispersibility studies, 1 mL of each formulation was taken and added to 500 mL of distilled water at 37±0.5°C and mixed at 50 rpm, and the in vitro performance of the formulations was evaluated visually using the grading system. To determine the self-emulsification time, 1 mL of SNEDDS was evaluated visually to the appearance of the final emulsion at 37±0.5°C in 250 mL of water at the mixing time at 50 rpm.

The % transmittance of the system forming the formulations was measured at a wavelength of 638 nm in a UV spectrophotometer using distilled water as a blank.

Droplet size and PDI measurements of nanoemulsions formed by adding 250 mL of water to the formulations were performed with Malvern Zetasizer Nano ZS at 25±0.5°C.

The effects of pH and liquid volume on droplet size and PDI were investigated for formulations containing no active ingredient and loaded with BOS by diluting them at enzyme-free pH 1.2, pH 6.8, and distilled water at 1:100, 1:250, 1:500 ratios.

Thermodynamic stability studies was included heating-cooling, centrifugation, freeze-thaw studies. The droplet sizes of the formulations containing no active ingredient and loaded with BOS

were also examined in terms of droplet size and PDI after dilution with 250 mL of water before and after the cycle.

Long-term stability, chemical and physical stability of BOS-loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations at 4°C, 25±2°C / 60±5% relative humidity (RH) and 40±2°C / 75±5%RH were evaluated under different storage conditions. Formulations were evaluated at 0, 1, 3, and 6 months for physical appearance, droplet size, and PDI.

After morphological studies, glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations, which did not contain active substances and was loaded with BOS, was diluted 1:250 with distilled water, mixed with a magnetic stirrer. The sample with 1% w/v phosphotungstic acid was dropped on the grid and kept at room temperature for 5 minutes examined with a transmission electron microscope (TEM).

In Vitro Dissolution Studies

The in vitro dissolution study was performed with bosentan containing reference product Tracleer® tablet (expiry date 10/20 and batch number IW067A0401), BOS-loaded glyceryl monolinoleate, and glyceryl monooleate SNEDDS formulations, and test conditions are given below. Dissolution studies were carried out according to the certain experimental conditions. 5 mL of the aliquots were withdrawn at predetermined time intervals, (15, 30, 45, 60, and 90 min) from the dissolution media and with an equal volume of fresh medium to maintain the total volume. The withdrawn samples were filtered using a 0.45 µm. Samples were analyzed by the validated HPLC method.

In vitro dissolution studies test conditions

Method	USP Apparatus II (Paddle method)
Dissolution media	1% SLS, FaSSIF, FeSSIF
Media volume	900 mL
Wavelength	220 nm
Speed	50 rpm
Temperature	37°C±0.5°C
Sampling intervals	15, 30, 45, 60, and 90 minutes
Analysis method	HPLC
Tablet – capsule sample	3

Results

The glyceryl monolinoleate SNEDD system containing 30 mg of BOS obtained comprised of 10.11% glyceryl monolinoleate, 80.90% polyoxyl 40 hydrogenated castor oil, and 8.99% caprilcaproyl polyoxyl-8 glycerides.

- 5 The glyceryl monooleate SNEDD system containing 28 mg of BOS obtained comprised of 10.11% glyceryl monolinoleate, 80.90% polyoxyl 40 hydrogenated castor oil, and 8.99% caprilcaproyl polyoxyl-8 glycerides.

Self-emulsification efficiency was evaluated by time to disperse and self-emulsify, and it was determined that both formulations spontaneously formed nanoemulsions when diluted with the
10 gastrointestinal environment. The formulations were self-emulsifying to form a transparent system in under a minute.

Glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations without the active ingredient, BOS-loaded glyceryl monolinoleate, and glyceryl monooleate SNEDDS formulations gave transmittance values over 99%, and the nanoemulsification efficiency of SNEDDS was
15 confirmed. The results showed that both formulations were transparent.

In glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations containing no active substance and loaded with BOS, the droplet size is below 50 nm, and the PDI is less than 0.2 (Table 1) (Figure 1).

20 **Table 1.** Droplet size and PDI results of glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations

	Without active substance		BOS-loaded	
	GML SNEDDS	GMO SNEDDS	GML SNEDDS	GMO SNEDDS
Droplet size	16.27±0.49	14.44±0.21	17.11±1.17	16.76±1.78
PDI	0.140±0.044	0.149±0.008	0.180±0.063	0.200±0.025

GML: Glyceryl monolinoleate; **GMO:** Glyceryl monooleate

For glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations without the active
25 substance and BOS-loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations, it was proven that pH and liquid volume did not differ on droplet size and PDI. The droplet size was below 50 nm, and the PDI was smaller than 0.2.

No physical changes were observed as a result of thermodynamic stability studies. The formulations were also evaluated for droplet size and PDI. The droplet size was below 50 nm, and the PDI was smaller than 0.2.

The developed formulations were found to be physically and chemically stable at 4°C, 25±2°C / 60±5% RH and 40±2°C / 75±5% RH for 6 months, and the results were shown. During stability studies, no change in physical appearances such as turbidity or precipitation was observed in BOS-loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations. When examined in terms of droplet size and PDI, it did not show any change and was stable for 6 months.

Morphological studies showed that nanoemulsions were formed and their structural integrity was not impaired by drug loading (Figure 2).

As seen in Figure 3A, in distilled water containing 1% SLS, the active substance was released more than 80% from of the commercial product, glyceryl monolinoleate, and glyceryl monooleate SNEDDS formulations within 15 minutes and all within 30 minutes.

As seen in Figure 3B in FaSSIF media, the commercial product was released about 33% of the active substance after 90 minutes, while the BOS-loaded glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations were released approximately 69% and about 83% within 15 minutes, respectively. At the end of 90 minutes, BOS-loaded glyceryl monooleate and glyceryl monolinoleate SNEDDS formulations released 95% and 96% of the active substance in their content, respectively. BOS-loaded glyceryl monolinoleate SNEDDS formulation increased the percentage of cumulative dissolved by 2.90-fold compared to the reference tablet in FaSSIF media. BOS-loaded glyceryl monooleate SNEDDS formulation increased the percentage of cumulative dissolved by 2.88-fold compared to the reference tablet in FaSSIF media.

As seen in Figure 3C in FeSSIF media, the commercial product released approximately 11% of the active substance after 90 minutes, while the BOS-loaded glyceryl monooleate SNEDDS formulation released 85%, and the BOS-loaded glyceryl monolinoleate SNEDDS formulation 86%. BOS-loaded glyceryl monolinoleate SNEDDS formulation increased the percentage of cumulative dissolved by 7.71-fold compared to the reference tablet in FeSSIF media. BOS-loaded glyceryl monooleate SNEDDS formulation increased the percentage of cumulative dissolved by 7.62-fold compared to the reference tablet in FaSSIF media.

Glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations containing bosentan monohydrate,

- Using glyceryl monolinoleate and glyceryl monooleate as oil
 - Determination of nanoemulsion size by water titration
 - Optimizing the formulations without active substance with the Box Behnken design Design Expert® program
- 5 - The droplet size of glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations that do not contain active ingredients should be less than 50 nm and PDI less than 0.2
- Optimized Glyceryl monolinoleate: Polyoxyl 40 hydrogenated castor oil: Caprylocaproyl polyoxyl-8 glycerides loading 30 mg of the active substance into the SNEDDS system
 - Optimized Glyceryl monooleate: Polyoxyl 40 hydrogenated castor oil: Caprylocaproyl polyoxyl-8
- 10 glycerides loading 28 mg of the active substance into the SNEDDS system
- BOS-loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations are characterized by their droplet size below 50 nm and PDI less than 0.2.

APPLICATION OF THE INVENTION TO INDUSTRY

- 15 BOS-loaded glyceryl monolinoleate and glyceryl monooleate SNEDDS formulations are used in the preparation of liquid drug forms by increasing the solubility and dissolution rate of bosentan, a BCS Class 2 drug with a solubility problem.

Explanation of Figures

- 20 **Figure 1.** Image of particle size and distribution. A- Glyceryl monolinoleate SNEDDS formulation, B- Glyceryl monooleate SNEDDS formulation

Figure 2. TEM images. A- Glyceryl monolinoleate SNEDDS formulation without BOS, B- Glyceryl monooleate SNEDDS formulation without BOS, C- BOS-loaded glyceryl monolinoleate SNEDDS formulation, D- BOS-loaded glyceryl monooleate SNEDDS formulation

- 25 **Figure 3.** Dissolution rate profiles of a commercial product of bosentan monohydrate, glyceryl monolinoleate, and glyceryl monooleate SNEDDS formulations (Error bars represent standard deviation).

A- 1% SLS distilled water medium recommended by FDA,

B- FaSSIF media,

C- FeSSIF media,

T1 = Commercial product (Tracleer®); T2 = Glyceryl monooleate SNEDDS; T3 = Glyceryl monolinoleate SNEDDS

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CLAIMS

1. A SNEDD system comprising bosentan monohydrate and long-chain mono and diglyceride mixtures, wherein it comprises glyceryl monolinoleate or glyceryl monooleate as long-chain mono and diglyceride mixtures.
2. A SNEDD system according to claim 1, wherein it comprises glyceryl monolinoleate as oil, polyoxyl 40 hydrogenated castor oil as surfactant, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant in the liquid SNEDD system.
3. A liquid SNEDD system according to claim 2, wherein the ratio of liquid SNEDDS components glyceryl monolinoleate as oil, surfactant polyoxyl 40 hydrogenated castor oil, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant is 0.9:7.2:0.8 (w/w/w).
4. A liquid SNEDD system according to claim 3, wherein the particle size of liquid SNEDDS is below 50 nm, and the polydispersity value is less than 0.2.
5. A liquid SNEDD system according to claim 4, wherein the particle size of liquid SNEDDS is between 10-50 nm, and the polydispersity value is in the range of 0.1-0.2.
6. A liquid SNEDD system according to claim 5, wherein the BOS-loaded glyceryl monolinoleate SNEDDS has a droplet size of 17.11 nm and a polydispersity index of 0.180.
7. A liquid SNEDD system according to claim 4, wherein 1 g of glyceryl monolinoleate SNEDDS comprises 30 mg bosentan monohydrate.
8. A process to obtain liquid SNEDD systems comprising Bosentan monohydrate according to any one of the claims 1-3, wherein the process comprises the steps of;
- Obtaining SNEDDS with glyceryl monolinoleate as oil, polyoxyl 40 hydrogenated castor oil as surfactant, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant with ternary phase diagram and determination of the area by titration with water,
 - Loading bosentan monohydrate into the SNEDDS system comprising glyceryl monolinoleate, Polyoxyl 40 hydrogenated castor oil, Caprylocaproyl polyoxyl-8 glycerides
9. A liquid SNEDD system according to claim 1, wherein it comprises glyceryl monooleate as oil, polyoxyl 40 hydrogenated castor oil as surfactant, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant.

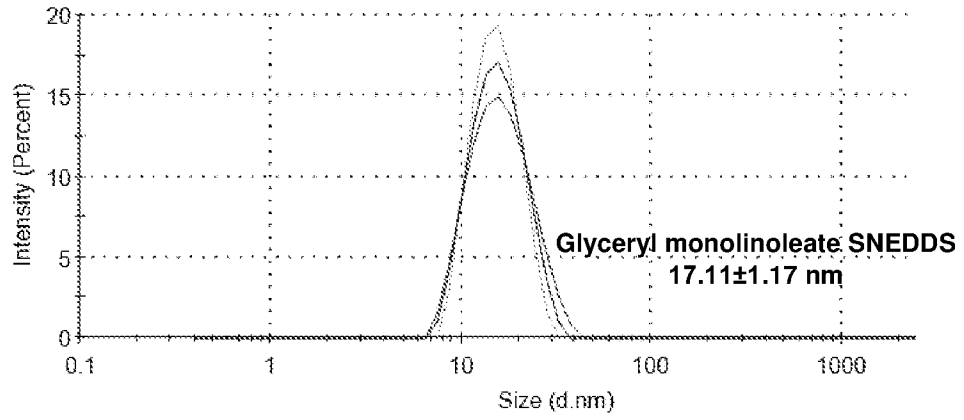
10. A liquid SNEDD system according to claim 9, wherein the ratio of liquid SNEDD components glyceryl monooleate as oil, polyoxyl 40 hydrogenated castor oil as surfactant, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant is 0.9:7.2:0.8 (w/w/w).
11. A liquid SNEDD system according to claim 10, wherein the particle size of liquid SNEDDS is
5 below 50 nm, and the polydispersity value is less than 0.2.
12. A liquid SNEDD system according to claim 10; wherein the particle size of liquid SNEDDS is between 10-50 nm, and polydispersity value is in the range of 0.1-0.2.
13. A liquid SNEDD system according to claim 12, wherein the BOS-loaded glyceryl monooleate SNEDDS has a droplet size of 16.76 nm and a polydispersity index of 0.200.
- 10 14. A liquid SNEDD system according to claim 10, wherein 1 g of glyceryl monooleate SNEDDS comprises 28 mg of bosentan monohydrate.
15. A process to obtain liquid SNEDD systems comprising Bosentan monohydrate according to claim 10, wherein the process comprises the steps of;
- Obtaining SNEDDS with glyceryl monooleate as oil, polyoxyl 40 hydrogenated castor oil as
15 surfactant, and caprylocaproyl polyoxyl-8 glycerides as co-surfactant with ternary phase diagram and determination of the area by titration with water,
 - Loading bosentan monohydrate into the SNEDDS system comprising glyceryl monooleate, Polyoxyl 40 hydrogenated castor oil, Caprylocaproyl polyoxyl-8 glycerides.

20

25

Figure 1

A



B

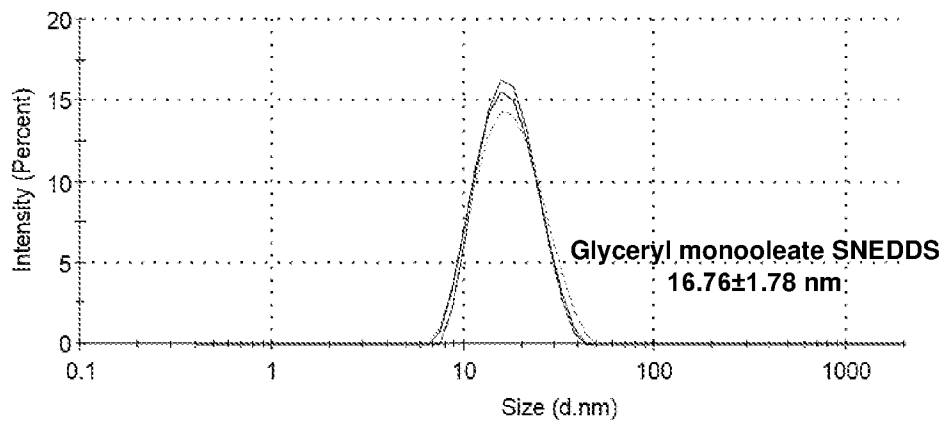
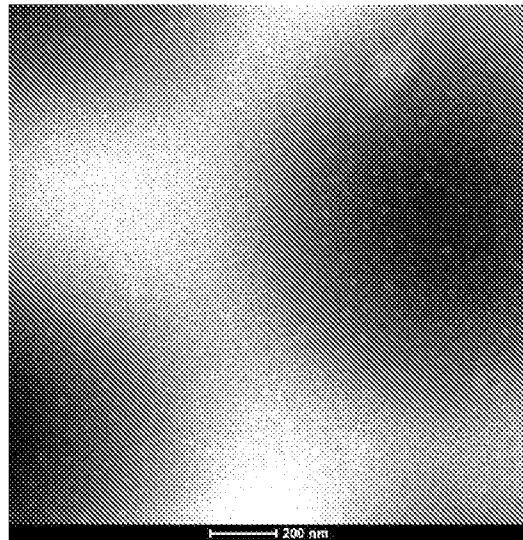


Figure 2

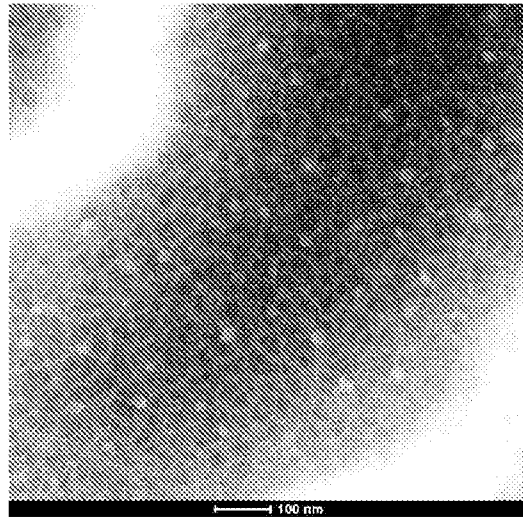
A



B



C



D

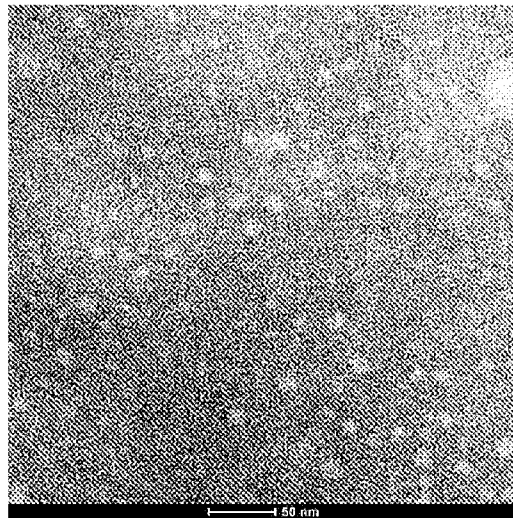
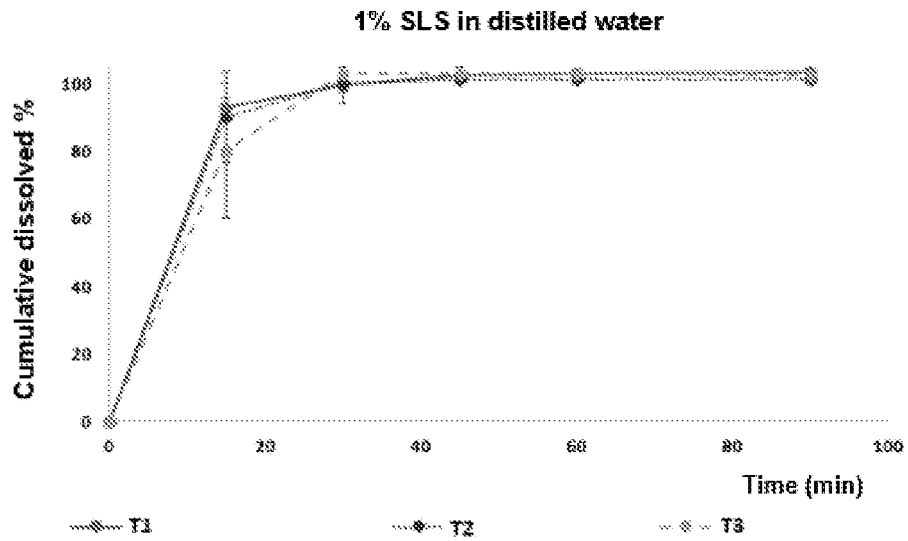
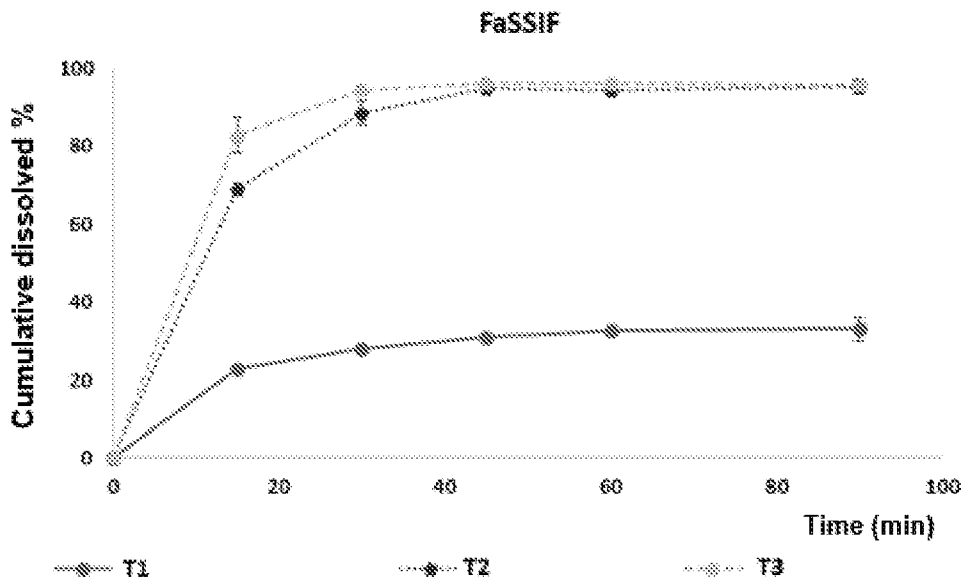


Figure 3

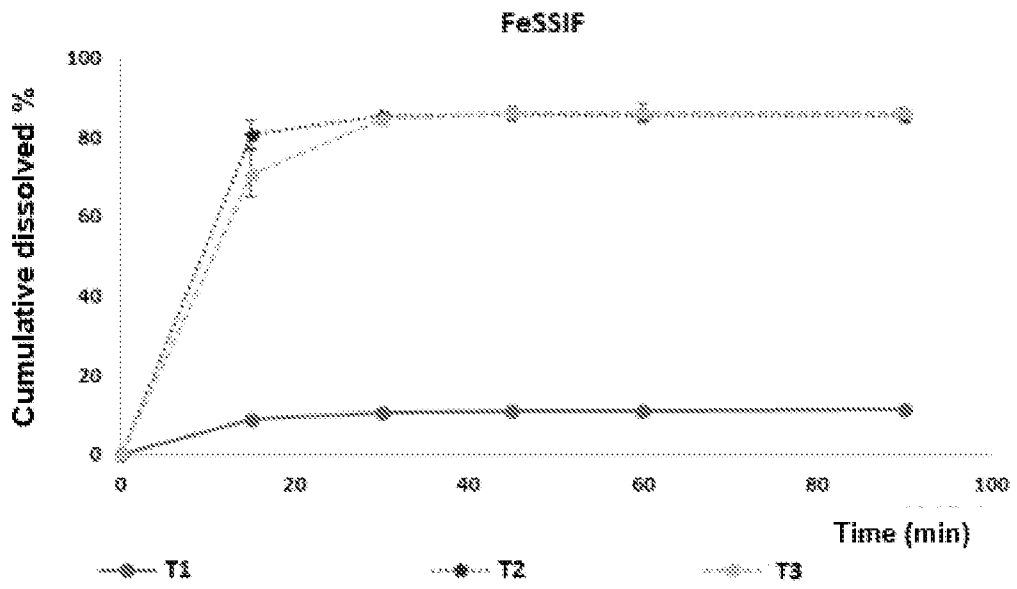
A



B



C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/TR2021/051258

A. CLASSIFICATION OF SUBJECT MATTER		
A61K 9/107 (2006.01)i; A61K 47/00 (2006.01)i; B82Y 5/00 (2011.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K 9/107; A61K 47/00; B82Y 5/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012139736 A1 (TIEFENBACHER ALFRED E GMBH & CO KG [DE]) 18 October 2012 (2012-10-18) Examples, claims	1-15
A	WO 2009095933 A2 (MSN LAB LTD) 06 August 2009 (2009-08-06) All document	1-15
A	KR 101058860 B1 (IND ACADEMIC COOP [KR]) 23 August 2011 (2011-08-23) All document	1-15
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 16 February 2022		Date of mailing of the international search report 16 February 2022
Name and mailing address of the ISA/TR Turkish Patent and Trademark Office (Turkpatent) Hipodrom Caddesi No. 13 06560 Yenimahalle Ankara Turkey Telephone No. +903123031000 Facsimile No. +903123031220		Authorized officer Nurgül SIKI Telephone No.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/TR2021/051258

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2012139736	A1	18 October 2012	EP	2696857	A1	19 February 2014
WO	2009095933	A2	06 August 2009	WO	2009095933	A3	30 June 2011
				EP	2240470	A2	20 October 2010
				EP	2240470	A4	23 May 2012
KR	101058860	B1	23 August 2011	NONE			