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BOSENTAN LOADED SOLID LIPID NANOPARTICLES: A RESEARCH

Prashant Patil[1], Komal Harak*[2] and Ravindranath Saudagar[3]

[1,2]Department of Pharmaceutics, R. G. Sapkal College of pharmacy, Anjaneri, Nashik- 422213, Maharashtra, India.

[3]Department of Quality Assurance Techniques, R. G. Sapkal College of pharmacy, Anjaneri, Nashik- 422213, Maharashtra, India.

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***Corresponding Author**
Komal Harak
Department of Pharmaceutics, R. G. Sapkal College of pharmacy, Anjaneri, Nashik- 422213, Maharashtra, India.

ABSTRACT

Solid lipid nanoparticles are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences. Due to their unique size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and tertiary levels of drug targeting. Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence have attracted wide attention of researchers. This review presents a broad treatment of solid lipid nanoparticles discussing their advantages, limitations and their possible remedies. The differenttypes of nanocarriers which were based on solid lipid like solid lipid nanoparticles, nanostructured lipid carriers, lipid drug conjugates are discussed with their structural differences. Different production methods which are suitable for large scale production and applications of solid lipid nanoparticles are described. Appropriate analytical techniques for characterization of solid lipid nanoparticles like photon correlation spectroscopy, scanning electron microscopy, differential scanning calorimetry are highlighted. Aspects of solid lipid nanoparticles route of administration and their biodistribution are also incorporated. If appropriately investigated, solid lipid nanoparticles may open new vistas in therapy of complex diseases.

KEYWORDS: Solid lipid nanoparticles (SLN), Objectives, preparation of aqueous dispersion of SLNs, Zeta potential, DSC, SEM, X-Ray differaction, In-Vitro drug release kinetics, Conclusion, References.

INTRODUCTION

Solid lipid nanoparticles

Solid Lipid Nanoparticles (SLN) are introduced in 1991, which is most developing formulations of nanotechnology with several applications in different fields like drug delivery, clinical medicine and research as well as in other varied science. SLN are the spherical particles of nanometer range which immersed in water or aqueous surfactant solution either using lipophilic or hydrophilic drug. Even the enhancement of solubility and bioavailability of poorly soluble drugs should be carried out using different biodegradable and bio acceptable polymers which can also overcome the toxic effects of traditional drug carrier system. Nano word derives from the Greek word '*Nanos'*, which means dwarf or extremely small.

Applications of nanotechnology in pharmaceutical field areas

a. **Nanosuspensions:** They are colloidal dispersions of nanosized drug particles that are produced by suitable method and stabilized by stabilizer.
b. **Nanoparticles:** They are solid colloidal particles size range from 30-100 nm.
c. **Nanospheres:** Polymer matrices in which drug is dispersed or dissolved.
d. **Nanocapsules:** Membrane wall structure with an oil core containing drug.[3, 4]

Formulation components of SLNs

General ingredients include the drug, solid lipids, emulsifiers, and water. Depending on the application, other ingredients might be present (osmotic agents, matrices for lyophilization, buffers, etc.). Table no. 3 mentions formulation ingredients for SLN preparation.

Table no. 1: Ingredients used in SLN formulation.[3]

Name of the ingredients	
Lipids	**Emulsifiers /Coemulsifiers**
Triglycerides Tricaprin Trilaurin Trimyristin Tripalmitin Tristearin Hydrogenated co-glycerides **Hard fat types** Witepsol W 35 Witepsol H 35 Witepsol H 42 Witepsol E 85 Glyceryl monostearate (Imwitor 900) Glyceryl behenate (Compritol 888 ATO) Glyceryl palmostearate (Precirol ATO 5) Cetyl palmitate Stearic acid Palmitic acid Decanoic acid Behenic acid Acidan N 12	Soyabean lecithin (Lipoid S 75,Lipoid S 100) Egg lecithin (lipoid E80) Phosphatidylcholine (Epikuron 170, Epikuron 200) Poloxamer 188 Poloxamer 182 Poloxamer 407 Poloxamine 908 Tyloxapol Polysorbate 20 Polysorbate 60 Polysorbate 80 Sodium cholate Sodium glycocholate Taurocholic acid sodium salt Taurodeoxycholic acid sodium salt Butanol Butyric acid Dioctyl sodium sulfosuccinate Monooctylphosphoric acid sodium

Objectives

1. Increase in solubility.
2. Increase in dissolution rate.
3. Reducing the dose of drug administered.
4. Formation of Solid Lipid nanoparticle.
5. Improve the pharmacodynamics and Pharmacokinetic.

Preparation of aqueous dispersion of SLNs

1. Drug loading

The aqueous dispersion of SLN formulation containing 2%w/w, 4%w/w, 6%w/w, 8%w/w, 10%w/w, 12%w/w and 14%w/w drug does not show any precipitation of drug while the formulation containing 16%w/w drug shows precipitation within 12 hr. Thus, in liquid formulation maximum 15%w/w drug can be loaded without any precipitation.

Table no. 2: Drug loading study of different Liquid SLN formulation.

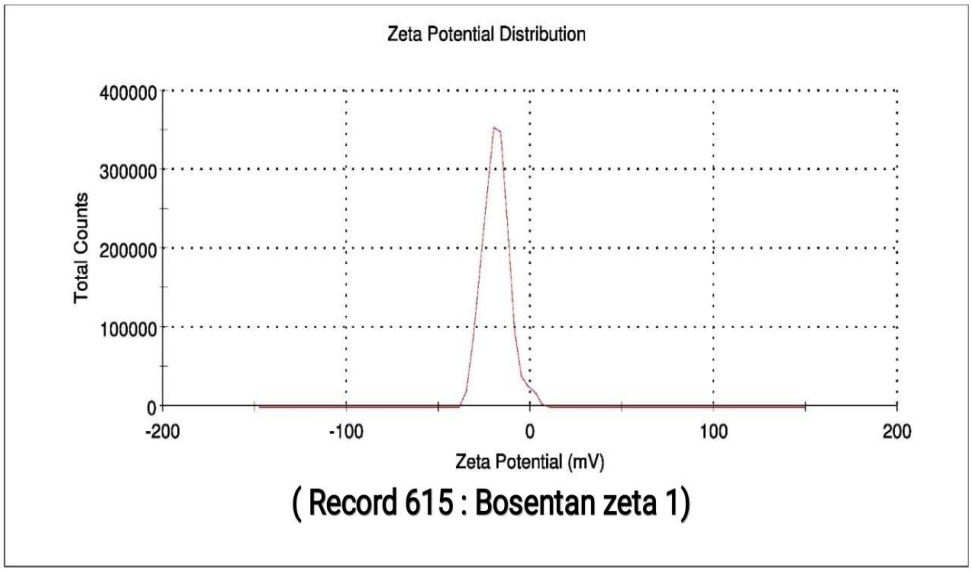
Sr.no.	Time (hr)	Q1	Q2	Q3	Q4
1	0	Clear	Clear	Clear	Clear
2	12	Clear	Clear	Clear	Clear
3	24	Clear	Clear	Clear	Clear
4	48	Clear	Clear	Clear	Clear
5	72	Clear	Clear	Clear	Clear
6	96	Clear	Clear	Clear	Clear
7	120	Clear	Clear	Clear	Clear

Table no. 3: Drug loading study of different liquid SLN formulation.

Sr. no.	Time (hr)	Q5	Q6	Q7	Q8
1	0	Clear	Clear	Clear	Clear
2	12	Clear	Clear	Clear	Crystal out
3	24	Clear	Clear	Clear	Crystal out
4	48	Clear	Clear	Clear	Crystal out
5	72	Clear	Clear	Clear	Crystal out
6	96	Clear	Clear	Clear	Crystal out
7	120	Clear	Clear	Clear	Crystal out

Table no. 4: Zeta potential measurement of liquid formulation.

Formulation	Zeta potential	Conductivity (mS/cm)
Liquid formulation (Q2)	-18.8	0.536



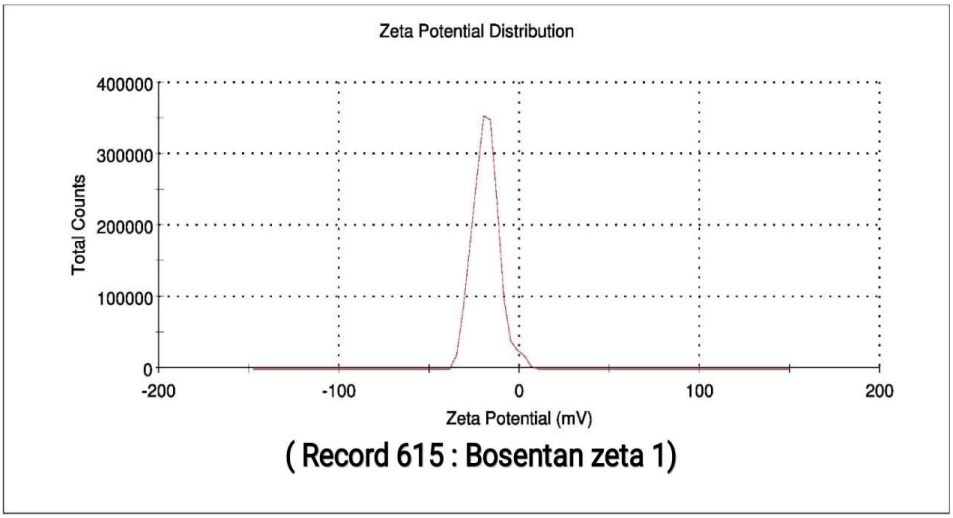


Figure no. 1: Zeta potential measurement.

Preparation of SLN (solidification)

As Q2 formulation passes all the evaluation parameter and also shows very good nanoparticulate property, it was selected as final formulation and it was converted into SLN. Liquid formulation (Q2) was converted in to solid lipid nanoparticle by spray drying technique using Aerosil 200 (Colloidal Silicon Dioxide) as a carrier. 10 g of liquid formulation contains 1500 mg of drug Bosentan. After addition of 5 g of Aerosil 200 as acarrier for spray drying the total weight 15 g contains 1500 mg of drug. Therefore by calculation 100 mg of spray dried powder contains 10 mg of Bosentan as a dose of drug.

Figure no. 2: Preparation of SLN by Spray drier.

Figure no. 3: SLN powder.

RESULT AND DISCUSSION

1. Organoleptic characterization of drug

Table no. 4: Organoleptic properties of drug.

Sr.no.	Parameter	Observation
1	Color	White
2	Odor	Odorless
3	Appearance	Solid crystalline powder

2. Melting point[101]

Melting point of bosentan was studied by capillary method.

Table no. 5: Melting point of drug.

Sr.no.	Method	Melting Point
1	Reference	107-110°C
2	Capillary method	105-111°C

3. Solubility study

Solubility of Bosentan was studied in different solvent like distilled water, 0.1N HCl, methanol by using shake flask method. Results are as follows.

Table no. 6: Solubility study of drug.

Sr. no.	Solvent	Solubility (mg/ml)
1	Distilled water	1.5
2	0.1N HCl	7.8
3	Methanol	5.6

Drug-Excipient compatibility study

Visual method: Drug-Excipient compatibility study was done for four week at 25°C (Room Temperature) and 40°C and samples are visually observed initially, after 2, 3, 4 weeks for any color change and results are shown in figure. The visual observation shows that there is no color change observed during storage for 4 week.

Table no. 7: Visual observation of drug and Excipients.

Sr.no.	Drug + Excipients	Temp.	Initially	1 week	2 week	3 week	4 week
1	Drug + Oleic acid	25°C 40°C	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid
2	Drug + Tween 80	25°C 40°C	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid
3	Drug + Cremophor RH 40	25°C 40°C	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid
4	Drug + Transcutol P	25°C 40°C	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid	Transparent liquid

Preparation of aqueous dispersion of SLN

2. Drug Loading

The aqueous dispersion of SLN formulation containing 2%w/w, 4%w/w, 6%w/w, 8%w/w, 10%w/w, 12%w/w and 14%w/w drug does not show any precipitation of drug while the formulation containing 16%w/w drug shows precipitation within 12 hr. Thus, in liquid formulation maximum 15%w/w drug can be loaded without any precipitation.

Table no. 8: Drug loading study of different liquid SLN formulation.

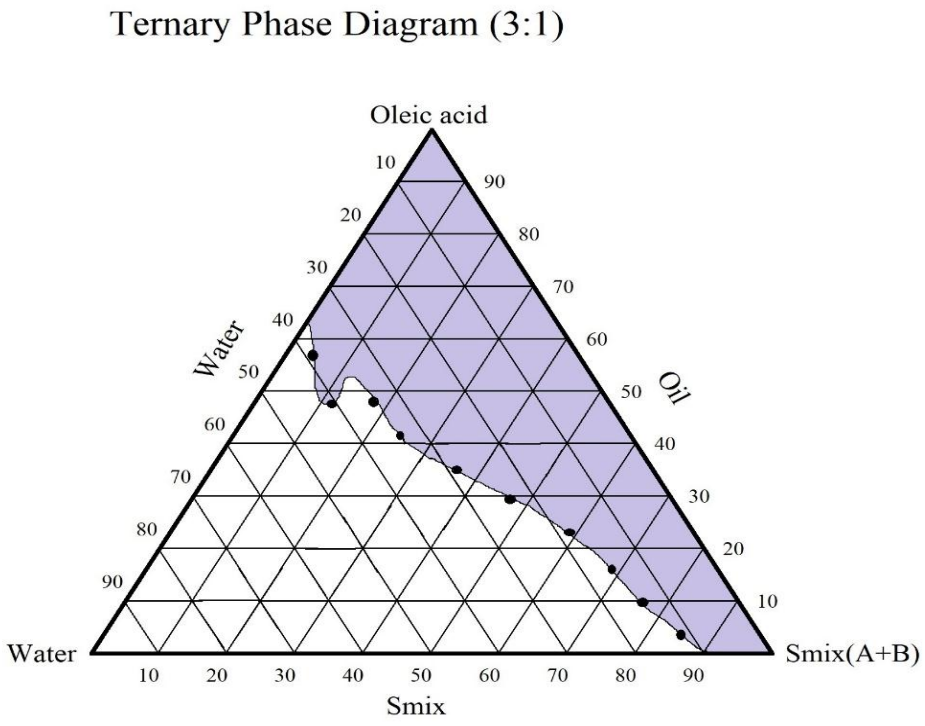
Sr. no.	Time (hr)	Q1	Q2	Q3	Q4
1	0	Clear	Clear	Clear	Clear
2	12	Clear	Clear	Clear	Clear
3	24	Clear	Clear	Clear	Clear
4	48	Clear	Clear	Clear	Clear
5	72	Clear	Clear	Clear	Clear
6	96	Clear	Clear	Clear	Clear
7	120	Clear	Clear	Clear	Clear

Table no. 9: Drug loading study of different Liquid SLN formulation.

Sr. no.	Time (hr)	Q5	Q6	Q7	Q8
1	0	Clear	Clear	Clear	Clear
2	12	Clear	Clear	Clear	Crystal out
3	24	Clear	Clear	Clear	Crystal out
4	48	Clear	Clear	Clear	Crystal out
5	72	Clear	Clear	Clear	Crystal out
6	96	Clear	Clear	Clear	Crystal out
7	120	Clear	Clear	Clear	Crystal out

1. Development of liquid SLN

Drug loading in liquid SLN formulation shows that maximum 15%w/w drug can be loaded in to the SLN formulation. Hence for further study different formulations containing 15%w/w drug were selected with the varying concentration of lipid, surfactant: co-surfactant (3:1). System with highest water absorption capacity was selected for further formulation and also system showing larger microemulsion region. The formulation prepared by selecting composition in the region showing in figure.



Drug content determination

Amount of drug present in the liquid formulation (table no. 10) was determined by UV Spectrometric method.

Table no. 10: Drug content of different liquid SLN formulation.

Sr. no.	Formulation code	Drug Content(%w/w)
1	Q1	93.59
2	Q2	96.86
3	Q3	95.25

2. Drug release study

In vitro drug release study was performed for Bosentan and liquid SLN. Results are shown in table no. 11.

Table no. 11: In-vitro drug release study of bosentan and liquid SLN.

Sr. no.	Time (min)		% DR	
		Plain Drug	Q1	Q2
1	5	0.696	42.318	45.372
2	10	3.269	58.068	60.436
3	15	9.754	69.049	76.083
4	30	15.965	75.877	81.196
5	45	17.475	88.642	89.740
6	60	21.696	96.294	96.843

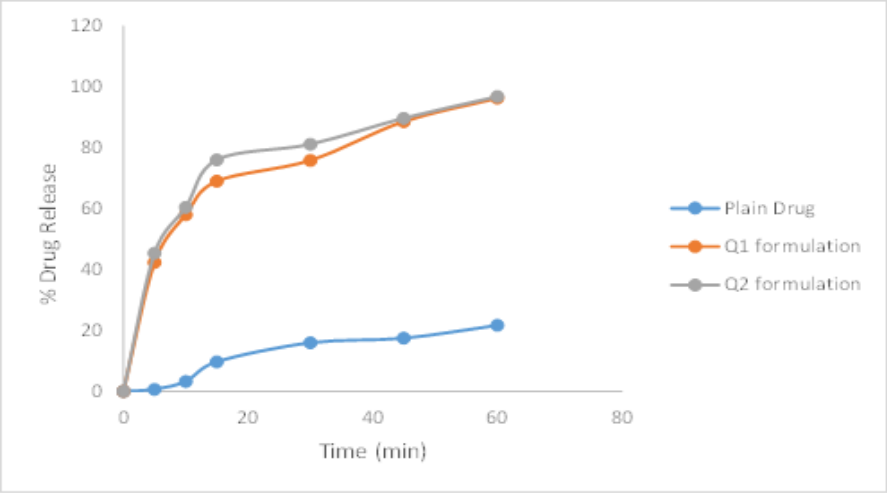


Figure no. 4: Comparison of dissolution profile of plain drug, liquid formulation (Q1 and Q2).

In vitro release study results revels that only 21.696%w/w drug was released from plain Quetiapine fumarate filled in capsule in 60 min while 96.294%w/w, 96.843%w/w drug release from the liquid formulation Q1, Q2 formulation respectively within 60 min.

Precipitation assessment

The formulated liquid formulation diluted with the 100 ml of distilled water and the diluted formulation observed for the precipitation and results was shown in Table no. 12.

Table no. 12: Precipitation assessment of different liquid formulation.

Sr. no.	Formulation code	Precipitation after 24 hrs.
1	Q1	Transparent, clear liquid, no precipitation, stable
2	Q2	Transparent, clear liquid, no precipitation, stable
3	Q3	Precipitation after 24 hrs.

From precipitation assessment Q1 and Q2 formulation found to be transparent, clear liquid with no precipitation and found to be stable. Q3 formulation forms whitish precipitate within 12 hrs.

Differential scanning calorimetry

DSC of Bosentan (plain drug) and its SLN were performed and results are shown in Figure no. 5 and 6.

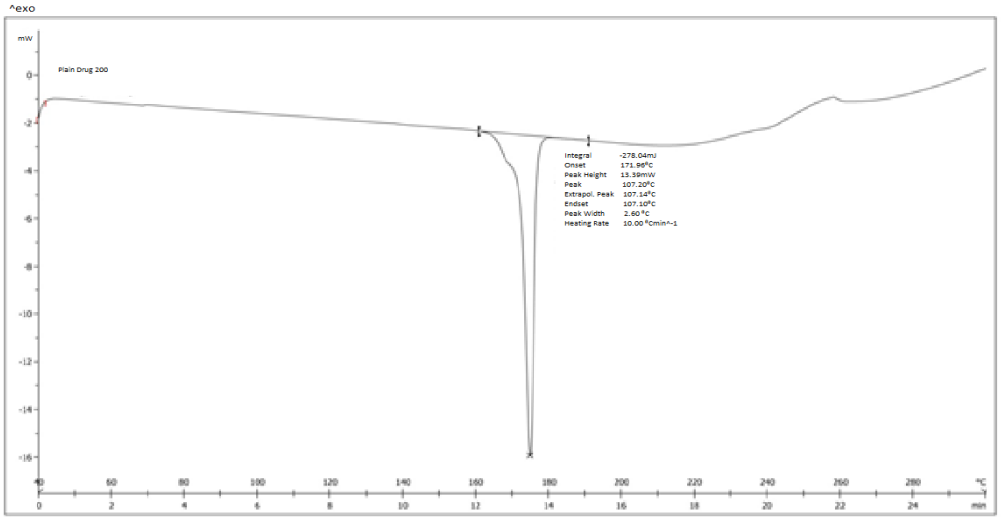


Figure no. 5: DSC spectra of bosentan.

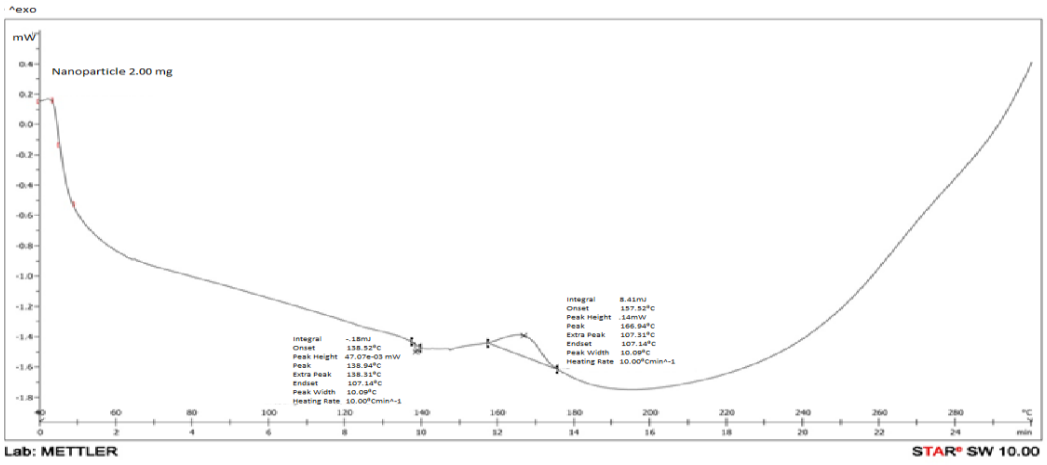


Figure no. 6: DSC spectra of bosentan SLN.

DSC of Bosentan exhibits a sharp melting point at 174.27°C with onset 171.96°C and end set or recovery at 176.54°C. The DSC of nanoparticle does not show the sharp peak. The absence of sharp melting peak indicates that the lipids and Aerosil 200 inhibits the crystallization of drug i.e. is in amorphous form or in solubilized form in SLN.

Scanning electron microscopy

Scanning Electron Microscopy (SEM) was used to determine the particle morphology of optimized SLN.

Results of Bosentan SLN was shown in Figure no. 7.

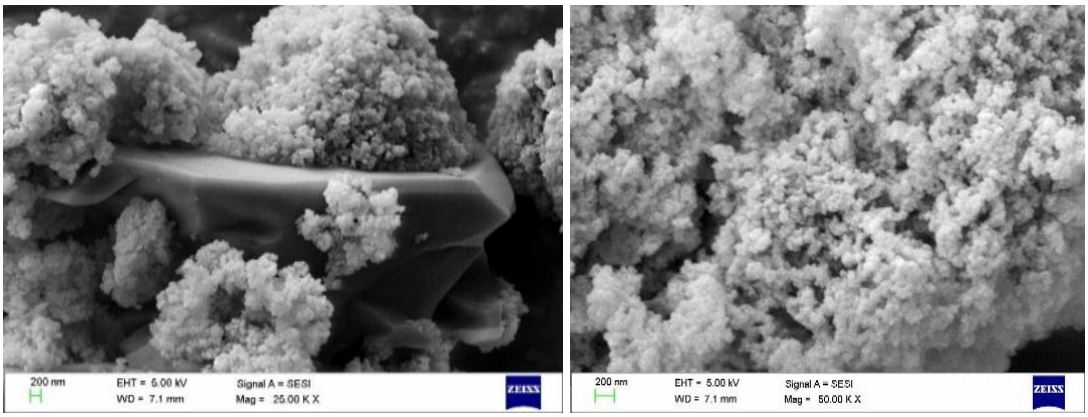


Figure no. 7: SEM images of bosentan SLN.

Powder X-ray diffraction

The X-ray diffraction pattern of Bosentan SLN was done and shown in Figure no. 8.

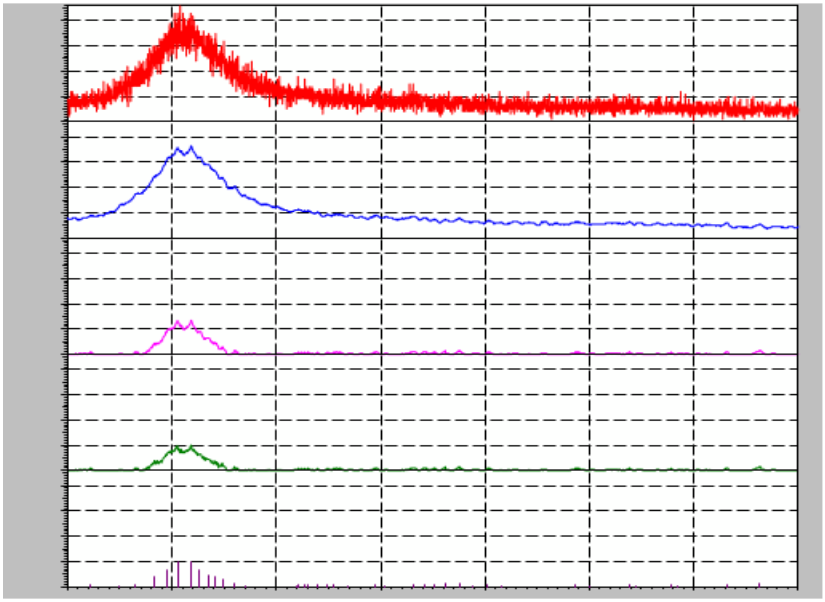


Figure no. 8: X-ray diffraction peaks of bosentan.

In vitro dissolution study

In vitro dissolution profile of Plain drug, liquid formulation, SLN, and Marketed formulation are compared together. SLN formulation shows more % drug release than the liquid formulation, marketed formulation and the plain drug.

Results of In vitro dissolution are displayed in Table no.13 and Figure no. 9.

Table no. 13: In vitro dissolution data of plain drug, liquid formulation, SLN and Marketed formulation.

Sr. no.	Time (Min)	% Drug Release			
		Plain drug	Liquid formulation	SLN	Marketed formulation
1	5	0.696	45.372	48.426	28.524
2	10	3.269	60.436	61.534	35.690
3	15	9.754	76.083	79.171	46.327
4	30	15.965	81.196	82.088	59.016
5	45	17.475	89.740	91.799	68.128
6	60	21.696	96.843	98.730	70.269

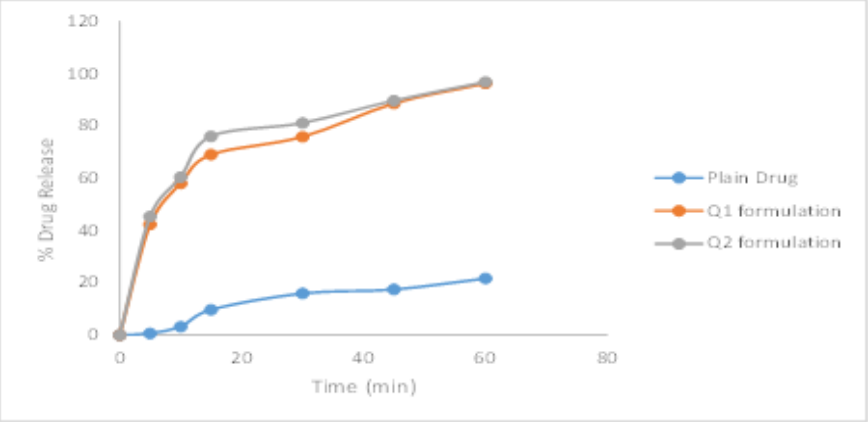


Figure no. 9: Comparison of dissolution profile of plain drug, liquid formulation (Q1 and Q2).

In vitro release study results revels that only 21.696%w/w drug was released from plain Bosentan filled in capsule in 60 min while 96.294%w/w, 96.843%w/w drug release from the liquid formulation Q1, Q2 formulation respectively within 60 min.

Release Kinetics of optimized formulation

1. First order

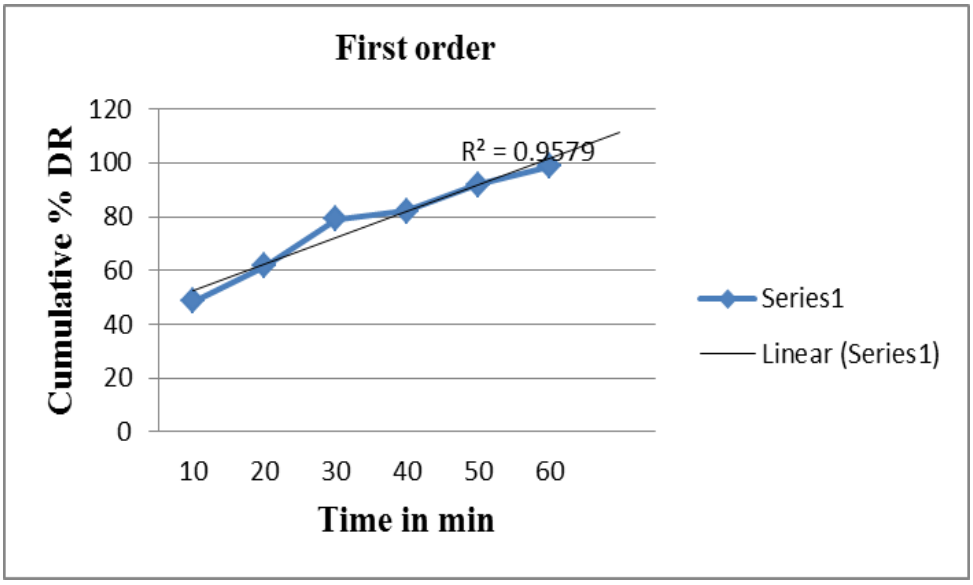


Figure no. 10: Release kinetic of Zero order SLN.

2. Higuchi model

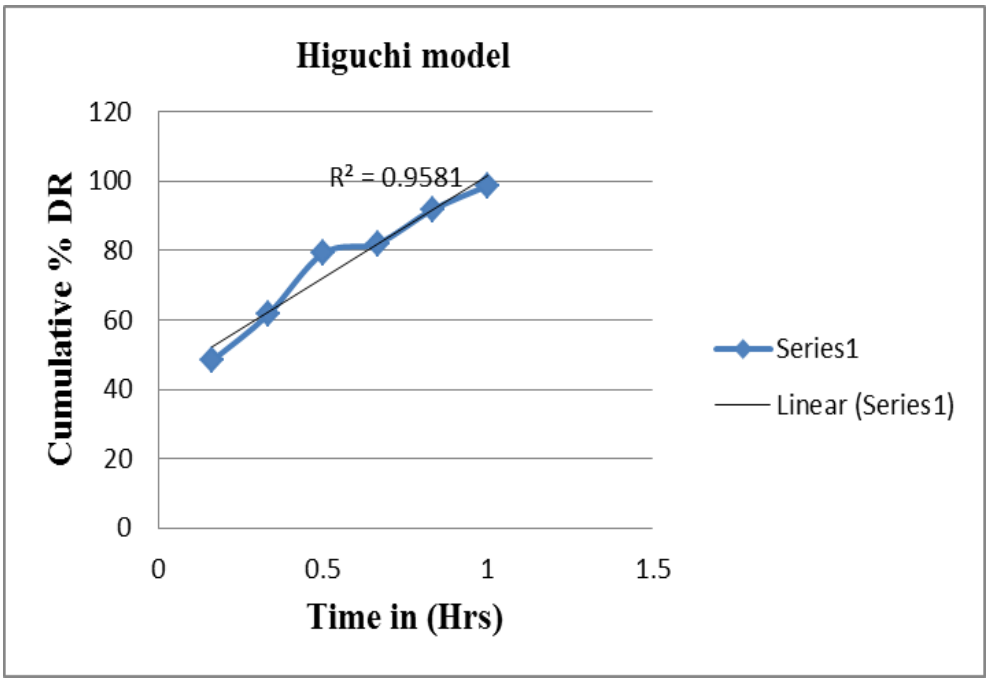


Figure no. 11: Release kinetic of higuchi model SLN.

CONCLUSION

This Study Concluded that Solubility study shows that Oleic acid as lipid, Tween 20 and Cremophor RH 40 as surfactatnt and Transcutol P as Co-surfactant shows good solubilizing property for Bosentan.

The Phase diagram shows that, increase in microemulsifying region with increase in the ratio of surfactant to co-surfactant from 3:1.

In-vitro release study shows that SLN can be used as possible alternative to conventional oral formulation of poorly aqueous soluble drug such as Bosentan, to improve its solubility and oral absorption.

Scanning Electron Microscopy, Differential Scanning Colorimetry, and powder X-ray Diffraction confirmed that the presence of Bosentan in a molecularly dissolved state in the SLN.

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