Advances in Bioresearch
Adv. Biores., Vol 11 (6) November 2020: 45-55
©2020 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.6.4555

Advances in Bioresearch

ORIGINAL ARTICLE

Fabrication & Characterization of Bosentan Loaded Solid Lipid Nanoparticles by 3^2 full factorial Design

[1.] Hitesh Katariya, [2]S. T. Prajapati
Assistant Professor, Smt. S. M. Shah Pharmacy College
Mahmemdavad, Gujarat, India -382415
[2]Kalol Institute of Pharmacy, Kalol, Gujarat, India- 382721
Corresponding author's Email ID: hitmin2008@gmail.com

ABSTRACT
The broad metabolism of Bosentan (BOS) with poor bio-availability (49 per cent) occurs first-pass (FPM). The BOS loaded solid lipid nanoparticles (SLN) formulation can prevent FPM's bypass. M-cells from peyer patches into the lymphatic system accumulate very lipidic BOS-SLN. As a strong lipid, Precirol 5 ATO was chosen. As the surfactant for preparation of SLN, Poloxamer188 was chosen. With the aid of the high speed homogenization process, the BOS-SLN was developed and configured with 32 complete factory designs. Using trehalose, BOS-SLN has been lyophilized. The tempo, time and sonic period of homogenization were also optimised (Process Parameters). BOS-SLN (dispersion & lyophilization) was done by a percent drug release analysis using Ph 7.4 (Apr0x. 94 percent drug release after 24 hours, higuchi model followed). In-vitro phosphate buffer phosphate bag system was used. Ex- Vivo permeability was conducted in the same environment as In-Vitro with chicken duodenum. Average particle sized (160 nm), percent drug traping (80.7 ± 1.6 percent), TEM (Sphere Types & Smoot Surface) and DSC-IR (Spheric Formulae), were used to classify BOS-SLN, which was stable for 45 days. Regulated releasing and stable drugs are seen by BOS-SLN.
Keywords: *Solid lipid nanoparticles, Bosentan, Colloidal drug carriers*

Received 12.09.2020 Revised 01.10.2020 Accepted 12.10.2020

How to cite this article:
H Katariya, S. T. Prajapati. Fabrication & Characterization of Bosentan Loaded Solid Lipid Nanoparticles by 3^2 full factorial Design. Adv. Biores., Vol 11 (6) November 2020: 45-55

INTRODUCTION

Bosentan has poor bioavailability (49 percent) because of massive volumes of FPM. Thus, FPM can be circumvented because of its nano scale by loading BOS onto SLN. Because SLN are highly lipid in nature, the M-cells of Peyer's bowel patch in the lymph system may easily be absorbed. BOS is extremely lipid in nature and is thus filled effectively in SLN. Therefore, FPM is avoided and Peyer's Patch is targeted, which may improve BOS bioavailability.

Nanoparticles made from polymeric polymers that are not biodegradable and are biodegradable. In siten-specific targeting and controlled release of inserted drugs, polymeric particles are commonly used. After cell internalisation, the cytotoxicity of polymers is important [1].

Moreover, it is challenging to manufacture large-scale polymeric nanoparticles. The focus of numerous research groups in the mid-1990s was on alternative nanoparticles made up of solid lipids, the nanoparticles called solid lipids. [2].

SLN combines the benefits of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) while at an equivalent time minimizing the associated problems of toxicity. [3] The anti-hypertensive agent Bosentan (BSN), is recommended. In the treatment of arteria pulmonalis hypertension, bSN is a dual endothelin-receptor antagonist. In the endo-A receptor (ET-A) and the endotheline-B (ET-B) receptor, the BSN is competitively antagonistic. This is extremely fatty (log P 2.9) [4].

BSN is water insoluble and is exposed to comprehensive metabolism first-pass (50 percent). The food channel cell lining consists of absorbent enterocytes of membrane epithelial (M) cells. Nanoparticles are

used in mixtures of endocytosis or transcytosis in cells that protect lymphoid aggregates called Peyer's patches [5].
Therefore, FPM can also be prevented by preparation of BOS-SLN, because the drug and lipid are very lipid. The bioavailability of Bosentan can be increased even in order to consume them through the lymphatic route and to bypass FPM [6].

MATERIAL AND METHODS

Materials
BOS, Dynasan 114, 116, 118, Sasol Germany, Compritol 888 ATO, Gattefose India (Pvt. LTD), Poloxamer 188, 47 (Hi Media Pvt LTd), Trehalose, Sigma Aldrich, Pvt. LTD (Gattefose India) Sample from Alembic Pharmaceutical, Vadodara), Precirolo 8 (Gattefose India Pvt Pvt LTD, Mumbai , India)

Methods

Preformulation Study
Melting point determination, FTIR, DSC, XRD, Solubility were done for Purity and compatibility study of drug and excipients.
Preparation of BOS- SLN
Due to its simple, reduced time consumption and efficacy, the high-speed homogenizer and ultra-sonication method was chosen. Precirol ATO 5 was melted above its melting point at 10 ° C and BOS was melted to lipid. Dissolved in twin purified water and dried up with the same temperature as the melting lipid, Poloxamer 188 (Surfactant). Shift to the BOS lipid mixture of heated aqueous surfactant solution. For the 10 minutes & 2 sonication periods, mixtures were homogenised at a high speed homogenised at RPM 15,000. For 10 minutes, a centrifuge of the SLN dispersion was used for lipid removal and unattached BOS at 8000 RPM. The SLN dispersion has been filtered by means of a pore scale 46 * 57 philtre. BOS-SLN containing supernatant has been reported
2.2.3 Optimization of BOS-SLN Precirol 5 ATO Lipid was chosen based on a solubility analysis and a coefficient of partitioning of BOS in lipids from different lipids (Dynasan 114,116,118, Compritol 888 ATO, and Precirol 5 ATO) [7].Poloxamer 188 Surfactant was chosen on the basis of the minimal Average Particle size and maximum percentage Drug Entrapment from both surfactants (Poloxamer 188 and Poloxamer 407). Method parameters such as sonic period, high-pressure homogenizer (HPH) rotation speeds and HPH rotation period were optimised via the test error set[8]. By 32 total factory experimental architecture, BOS-SLN was designed. Formulative parameter such as drug: the lipid ratio and surfactant concentration are different variables and the MPS are dependent on the Efficiency of the Trapping (EE percentage) percent. A check-point analysis was conducted to validate that the polynomial equation and contour plots extracted play a role in prediction of responses to solid lipid nanoparticle [9].
Differences of the dependent variable the orally calculated values and the mean values of the experimentally collected dependent variable values have been monitored with t-test, which indicates that both values are identical to each other since $p > 0.5$ does not have a meaningful difference. Optimization has been made in order to define the degree of independent variables (X1 and X2), resulting in an overall particle size of maximum percentage EE and minimal. For the optimisation of the programme formulation, desirability was used.

Factors	Level		
	-1	0	+1
Drug: Lipid Ratio	1:10	1:15	1:20
Surfactant Concentration	1%	2%	3%

Table 1 independent Parameter with Levels

Lyophilization Process of BOS-SLN
Freeze drying approach was used to increase the stability and the leakage of Entrapped BOS in stable lipid nanoparticles.[10]. Absolute solid contents ration: the concept of average particulate size and drug trap (tréhalose) (1:3, 1:5) was introduced for optimization and chosen. (Table 6). Table 6. The final BOS-SLn lot was freezed and cryoprotacted using trehalose (1:5 = solid material in BOS-SLN: trehalose) for calculation and form preservation.[11]
Characterization of BOS-SLN
Particle size analysis
The formulations were studied by a Malvern zetasizer and Laser Beam Duration 2.40 mm, R.I: 1.456 Particle Scale Distribution of the formulations [12].
% entrapment efficiency (%EE)

The BOS loaded lipid nanoparticles have been isolated from free BOS by cooling centrifugation at 8000 rpm for 10 min to create a drug-trapment BOS percentage for solid lipid nanopartulate. The isolation, treatment and analyses with UV Spectrophotometry of free lipids and drugs that were developed. The utility of the drug entanglement of each lot was calculated by the following equation[13].

% EE = (Amount of entrapped drug into solid lipid nanoparticles/ Total Amount of drug)*100

Zeta Potential

Zeta potential is used for measure the physical stability of colloidal dispersions.

FTIR & DSC Study

FTIR spectrum of freeze dried BOS-SLN was obtained on Nicholet 6700 FTIR instruments. DSC study was performed to characterize physical state of BOS in SLN. DSC was performed by using differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan). Nitrogen gas was transported through the DSC chamber at a rate of 50 ml/min.

Transmission electron microscopy

Transmission Electron Microscopy (TEM) was conducted for morphological studies of BOS-SLN in the optimum environment.

In vitro Study

Drug release study

The customary dialysis bag approach assesses in vitro release of the BOS-SLN drug. One end was connected to the thread and inspected for leakage (Molecular weight cut 10000–12000).[14,20].

The 2.0 ml dispersion was completely packed with 1 mg-like scatter and thread was connected with the remaining open end. The bag was used as a donor bag. The dialysiological bag was submerged in a 7.4-ml phosphate buffer containing 37 ° C ± 2 ° C as the receiver bag. The beaker material was blended with an aluminium foil and the beaker was also sealed. Samples were extracted from the dissolution medium at selected intervals and replaced by a similar quantity of freshly prepared dissolution material. A UV spectrophotometer for BOS was tested at 271 nm, thereby measuring the percentage of drug release. To know the mechanism of the BOS-SLN release of drugs, models like zero order, the first order and Higuchi, Hixson and Peppas applied to drug release results. The same technique has been used to acquire freeze dried SLN drug release results.

Ex vivo study

Chicken duodenum was used for the slaughterhouse permeability and release analysis. Immediately the intestine was extracted and put in the Ringer buffer with ice-cold bubbles. The fabric was rinsed with an ice-cold regular ring tank to extract luminous material and dig segments[15]. Dispersion was then inserted into a duodenum on the one side of the bowel. There was also tied on the other side of the intestine. Aerated, the fabric was put in a buffer of 100 ml of phosphate 7.4 at 37 ± 0.5 ° C. At the predetermination interval, samples were extracted from the receptor compartment and replaced by the same buffer number. UV-Visible spectrophotometer samples were tested for drug content of 271 nm[16].

Stability study

Stability study of BOS-SLN dispersion

The stability analysis was carried out on optimised batch over a time span of 45 days, and stabilisation was conducted through parameters such as MPS and percentage of drug traps, accelerated state (40 ° C±2 ° C and 75% ±5% RH).[17,18,19]. At the initial period of 15 days, 30 days and 45 days, checks were conducted on all parameters for the configured batch.

Stability study of freeze dried BOS-SLN

Stability analysis of freeze dried BOS SLN was done in an accelerated 45-day environment (40 ° C ± 2 ° C and 75% ± 5% RH) and the parameters including MPS and percent drug trapping were conducted in stability tests.[18,20]. At the initial period of 15 days, 30 days and 45 days, checks were conducted on all parameters for the configured batch.

RESULTS AND DISCUSSION

Preformulation study

The melting points of drugs and excipients are in line with standards such that the melting point is not modified. The BOS and excipients FTIR spectrum shown in the Fig. 1. BOS and excipients functional group peaks were contrasted with norms that display no noticeable increases in frequency. Consequently, the inference is always that the BOS and excipients obtained were in pure shape. DSC BOS and excipients are seen in the melting point range to demonstrate the sharp endothermic peaks (see Fig . 2). When scanned in a UV-visible wavelength range from 200 to 400 nm, BOS in the methanol provides feature function. It is thus always concluded that the BOS and excipients strictly obtained. BOS and excipients DSC thermograms have both seen to be reliable since there is a sharp endothermic peak. At a wavelength of

271 nm it provides the maximum absorption. It is thus always assumed that the BOS and excipients procured were pure and consistent.

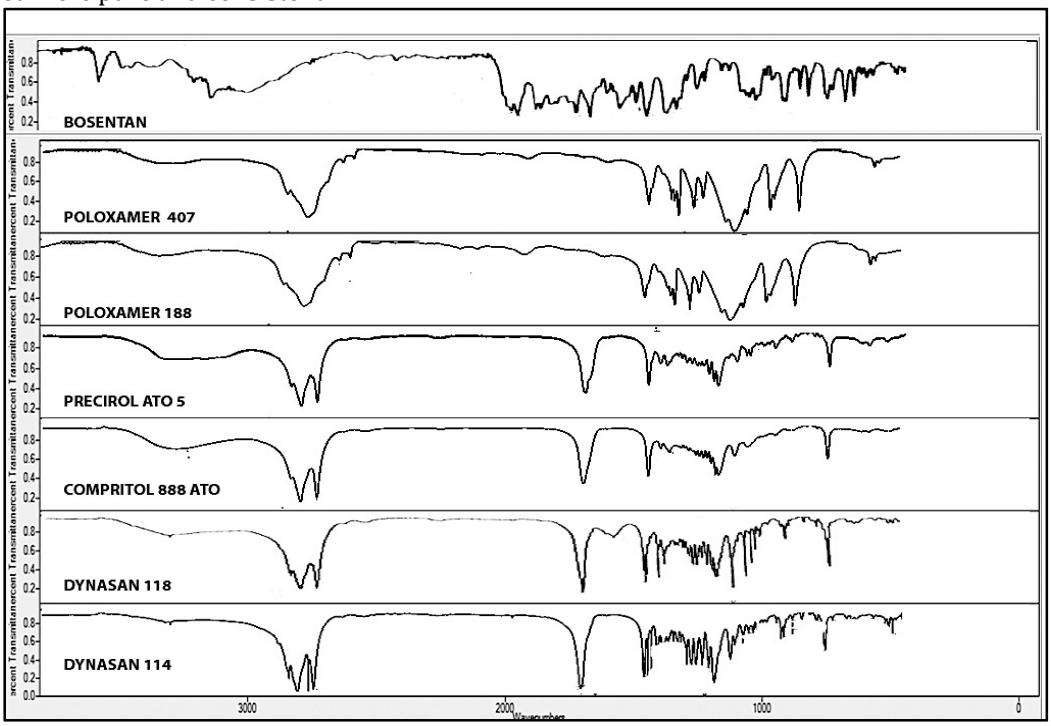


Figure: 1 FTIR spectrums of BOS & Excipients

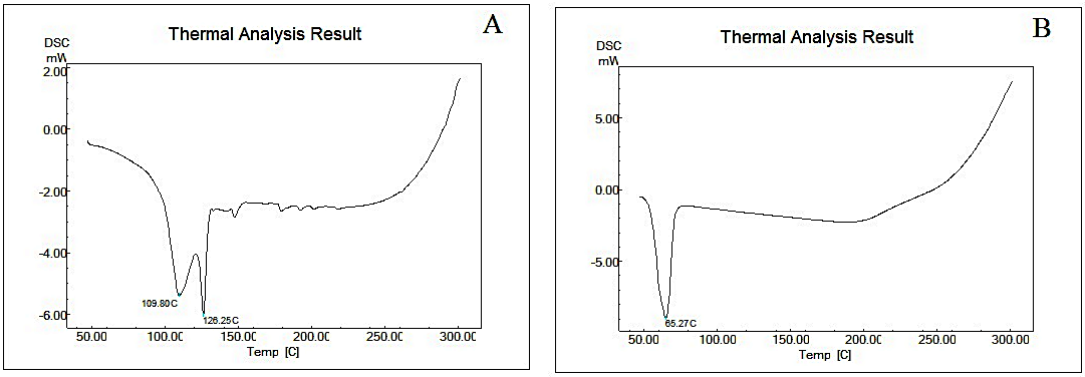


Figure 2 DSC Graphs of BOS and Precirol ATO 5

Optimization of BOS-NLC

Optimized process value by keeping minimum MPD, optimum percent Drug Entrapment requirements, 2 min Soning Time, 15 000 RPM High Speed Homogenizer, 10 min High Speed Homogenizer stirring period, Optimized process parameters efficiency.(Table 2).

Parameter		**Particle Size***	**% Drug Entrapment***
Sonication Time(min)	1min	180.28±1.060	65.62±2.81
	2min	**146.12±2.070**	**72.65±0.31**
	3min	141.67±2.170	56.79±0.65
For optimization of Sonication Time, HSH speed 15,000 RPM and HSH Time 10 min was kept constant			
HSH Speed (RPM)	10,000	175.42±1.260	67.32±1.34
	15,000	**150.75±1.270**	**74.62±0.87**
	17,000	158.23±2.320	61.12±0.36
For optimization of HSH speed, Sonication time 2min and HSH Time 10 min was kept constant			
HSH Time (MIN)	7min	175.4±1.76	62.23±0.56
	10min	**149.43±1.23**	**73.67±0.22**
	15min	152.45±0.64	68.34±0.98
For optimization of HSH time, Sonication time 2min and HSH speed 15,000 RPM was kept constant			

Table 2 Optimization of Process parameter

Design Professional 10.0.0 Program has been used to construct a mathematical model and hence the resulting statistical analysis for each response parameter. In order to create a relationship between the dependent variables and hence the dependent variables, the statistical analysis design was used. With the aid of the architecture professional, tests can be coordinated, info evaluated and the findings graphically represented. The results were analysed statistically at the 5 % significance level. Based on the p values, the best models have been chosen and the significance of that model has been inferred. In addition to testing the significance of variables, one-way analysis of variance. Also, the outcome was not completed by the F value and the P value of the model. For impact analysis of each attribute on the designed answer, a design expert was using 10.0.0 (State-Ease Inc. Minneapolis, USA). ANOVA used the polynomial equation to evaluate the statistical importance of the difference in component size and percentage opioid entrapment.

$$Y1\ (PS) = b0+b1X1+b2X2+b1^2X1^2+b2^2X2^2+b12X1X2$$

$$Y2\ (\%\ Drug\ Enrapment) = b0+b1X1+b2X2+b1^2X1^2+b2^2X2^2+b12X1X2$$

In the event that the vector Y1 or Y2 are the dependent, PS is the percentage EE, while b0 is intercept, and bij is the (b1) and bij (b12 is the polynomial secondary regression coefficient; and Xi is the independent formulant variable amount, X1 is the medicinal product: lipid, X2 is surfactant.

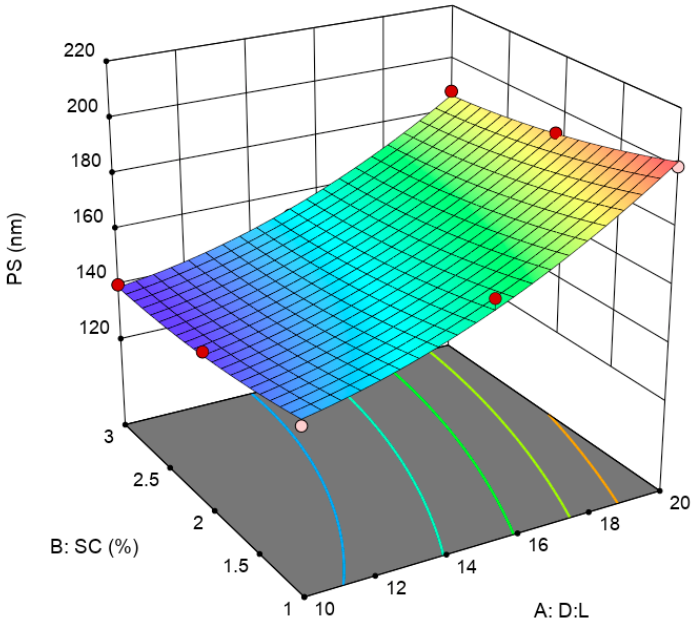
Batch No.	A	B	Y_1 (nm)*	Y_2 (%)*
1	-1	-1	144.6±5.30	75.11±4.14
2	-1	0	170.7±4.35	85.18±3.18
3	-1	+1	200.8±6.65	87.34±4.69
4	0	-1	141.7±09.72	70.21±3.08
5	0	0	156.8±3.80	77.34±2.18
6	0	+1	191.9±7.80	83.24±3.16
7	+1	-1	140.2±12.05	67.17±8.55
8	+1	0	152.8±4.21	74.12±5.22
9	+1	+1	187.9±10.55	78.12±3.38

Table 3 Values of MPS and % EE of BOS-SLN as per 3^2 full factorial designs

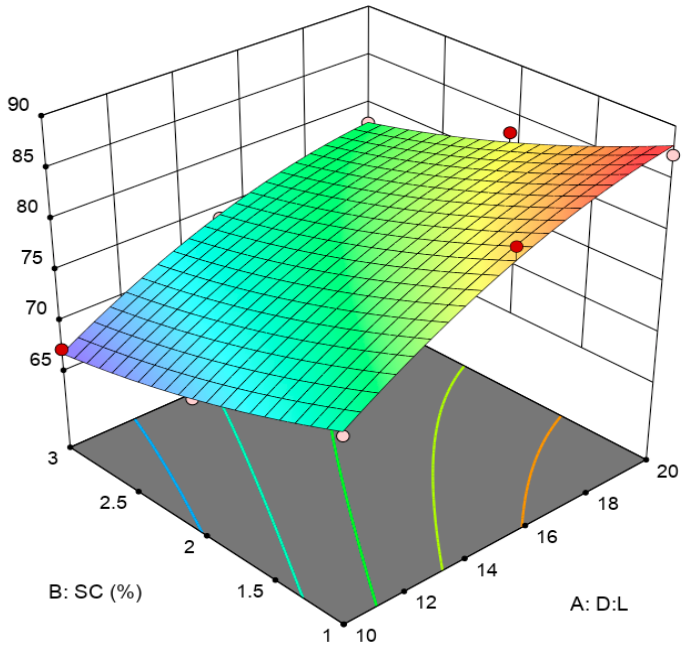
The PS values for 9 batches showed a large range from at least 140,2 nm to 200,8 nm and the variance from at least 67,17% to at least 87,34% for PDE. In SLNs at +1 level of X1 (1:20) and -1 level of X2 (1%), important low PS (140.2 nm) with high PDE (87.34), were obtained.

$$Y1 = +158.30 +25.68*A -5.87 *B -2.13*A*B +7.75*A^2 +2.70*B^2$$

$$Y2 = 78.27+6.04*A -4.70*B-0.32*A *B - 2.02*A^2+ 0.91*B^2$$



A



B

Figure 3 - 3D Response surface plots of optimized BOS-SLN for (A) Average Particle Size (B) % Drug Entrapment.

Effect of drug: lipid ratio and surfactant concentration on particle size
In Figure 3D Reaction Counter plot is seen for the distribution of particle size. 3. The graph indicates that a high MPS has been used in rising the ratio of medications and lipids and the concentration of surfactants due to the high amount of drugs and lipids.
Effect of drug: lipid ratio and surfactant concentration on % Drug Entrapment
The plot counter is shown in Figure for percent drug entrapment. 3. The graph indicates that, due to the high drug and lipid content, a high percentage of EE was observed as drug / lipid ratio and surfactant levels increased.
Check point analysis and desirability factor
In order to prove the forecast, a checkpoint analysis was carried out to validate the equation explaining the effect of the parameters on the variables. Two batches from checkpoints were produced and shown in Table 4. Predicted and actual MPS value and compared to me. After observing the impact of independent variables on the answers, the desirability function was used to maximise the most productive batch and to evaluate the number of variables that give the best answers.
The optimized batch with level of various factors, results and desirability is shown in Table 5.

Check Point Batch	Measured Value		Predicted Value	
	Particle Size	% drug entrapment	Particle Size	% drug entrapment
CP1	167.2±4.7	73.67±4.2	165.02	77.17
CP2	159.2±5.8	74.42±1.35	152.75	78.18

Table 4 Observation of Check Point Batch

Drug :lipid ratio	Surfactant concentration	Particle Size	%drug entrapment	Desirability
1:13	1%	160	80.70	0.78

Table 5Desirability of optimized batch

For trehalose concentration 1:3			
Before		After	
Particle Size	%drug Entrapment	Particle Size	% Drug Content
162.7 nm	79.56%	188.5 nm	65.98 %
For trehalose concentration 1:5			
Before		After	
Particle Size	%drug Entrapment	Particle Size	%drug Content
162.7 nm	79.56%	178.5 nm	76.12 %

Table 6 Optimization of lyoprotectant

Characterization of BOS-SLN

The BOS-SLN batch is designed with 157.1 nm MPS (Fig . 4). The percentage EE of 69.58 to 97.52% was noticed. The configured lot displays a cumulative EE of 80.70±1.6%. The optimised batch potential -36.6 mv (Fig . 5) was obtained. The system's steadiness is improved as the zeta potential is more pessimistic. The steric stabilizers and carboxylic BOS group have low zeta potential. FTIR BOS and Freeze dried BOS-SLN continuum showed that the FTIR Optimize Batch continuum had a major shift from the FTIR drug spectrum of practical group frequencies (FIR 6). The BOS and Precool ATO 5 endothermic sharp peak at 126.250c, 65.270c respectively was observed. Owing to tiny and uniform particles in the BOS SLN, wide and tiny endothermic peak freeze BOS SLN was observed at 65-310c.Results are shown in Figure 7.

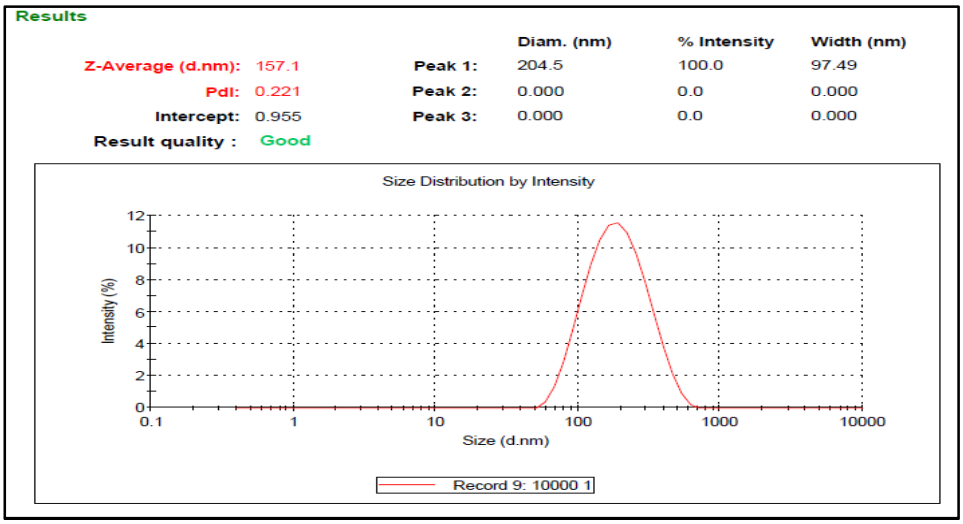


Figure: 4 Particle size distribution

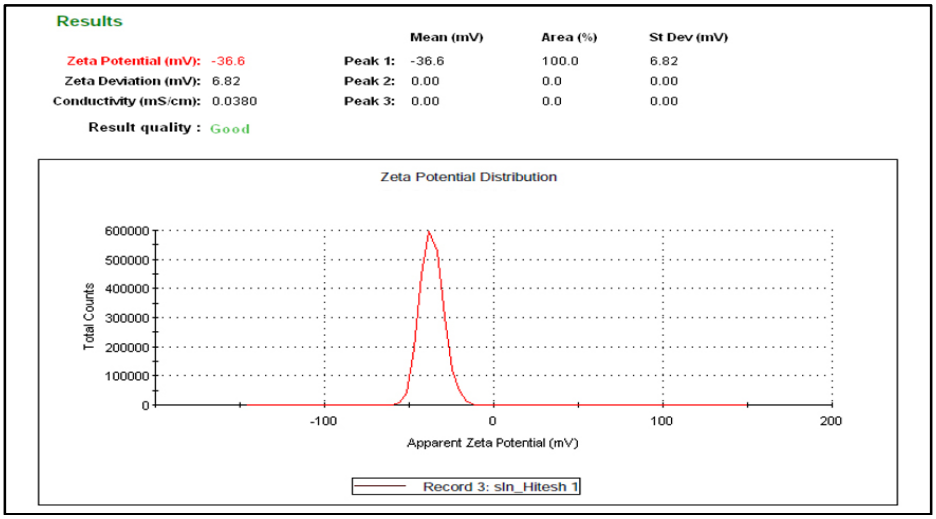
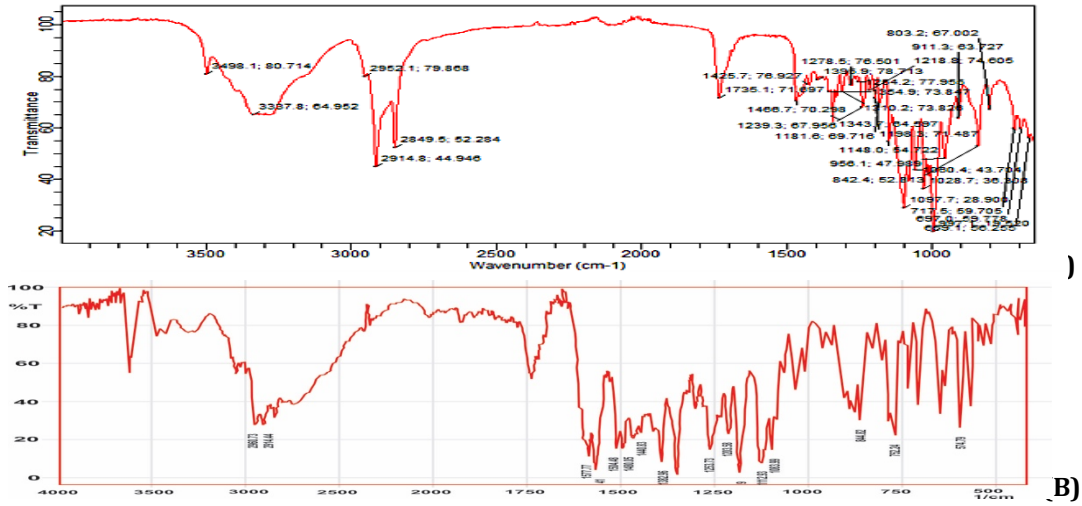


Figure: 5: Zeta potential distribution



)

B)

Figure6 FTIR Spectrum of Freeze dried BOS loaded SLN (A) & Bosentan (B)

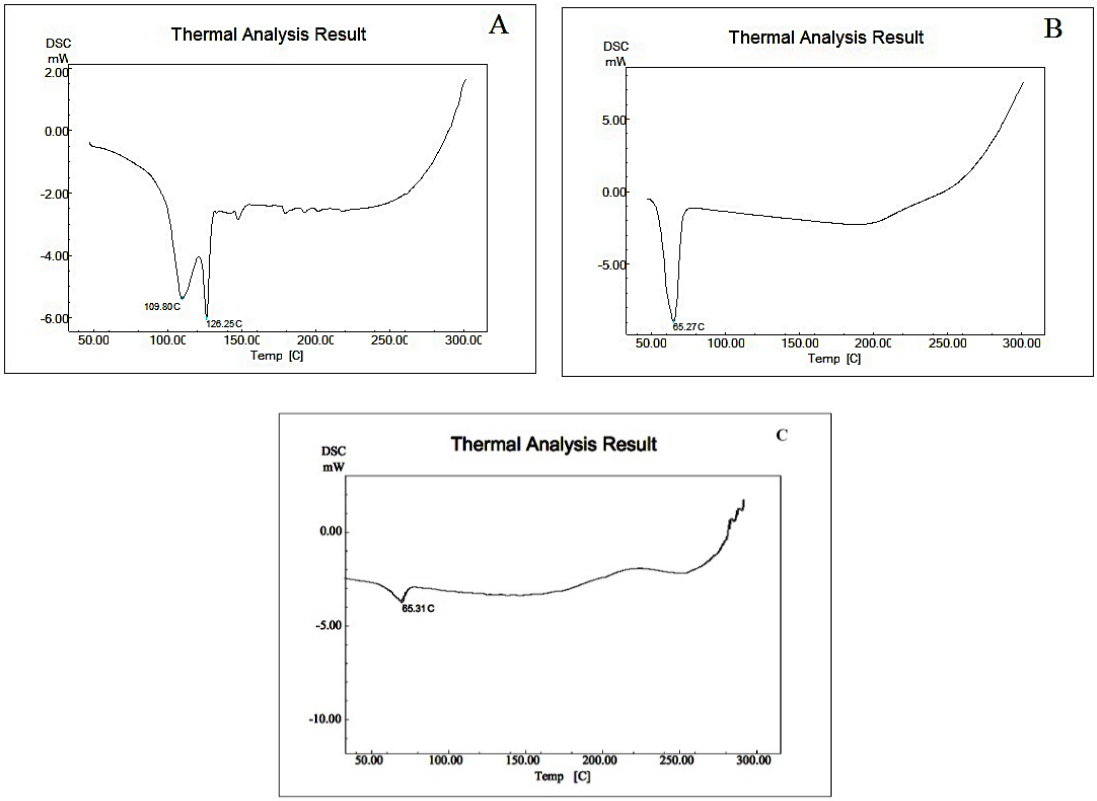


Figure 7 DSC Thermogram of BOS, Precirol 5 ATO and BOS SLN

Transmission electron microscopy

The TEM picture of BOS-SLN indicates that particles have a smooth surface in spherical form without the rough spores seen in the Fig. 8 The exterior layer consists of an inside and surfactant lipid matrix which was advantageous for the drug's continued production.

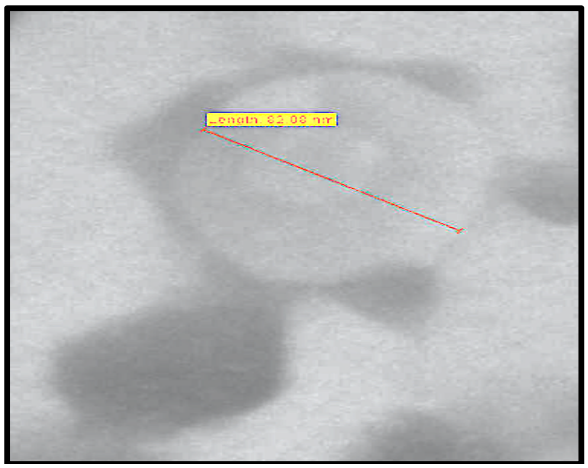


Figure 8. TEM Image of optimized BOS-SLN Batch.

In vitro study

Medicinal phosphate dissolution buffer 7.4 was used in vitro drug release to validate release patterns of strong lipid nanoparticles. Table No:7 indicates the total percentage of accumulated BOS-SLN drug release for optimised batch. The optimised formula showed an initial release of BOS SLN Dispersion of 15.06 ±2.65 per cent within the first hour. Further dissolution in the pH7.4 phosphate buffer up to 24 hours and percent CDR achieved after 24 hours were found to be in 94.32, which suggests a consistent liberation of BOS from BOS SLN. Durable release of BOS into lipid, compact and uniform particle and strong matrix of the compound was obtained because of the high partition of BOS. Data on opioid release were used for various models of dissolution. Comparison of different dissolution models as seen in Table 8. In vitro drug release follows higuchi model. So drug release occurs accordingly Fick's law.

Time	%CDR (I)	%CDR (II)	%CDR (II)	Average + S.D
0	0	0	0	0.00
1	12.54	17.15	17.13	15.06 ± 2.655
2	25.14	24.06	23.83	24.33 ± 0.699
4	36.05	35.17	32.51	34.57 ± 1.843
6	45.12	44.32	4747	45.63 ± 1.632
8	51.12	53.80	55.25	53.39 ± 2.09
10	62.65	63.03	64.89	63.52 ± 1.19
12	73.06	74.87	75.52	74.48 ± 1.27
16	77.34	79.54	80.12	79.0 ± 1.466
24	94.79	93.12	95.05	94.32 ± 1.04

Table 7 *In vitro* drug release data of dispersion of BOS SLN

Model	**R^2**	**K**
Zero Order	0.9020	3.18
First order	0.9926	0.091
Higuchi	**0.9864**	**19.48**
korsemeyer peppas	0.9895	17.4
Hixon crowell	0.9793	0.029

Table 8 Comparison of various dissolution models for Dispersion BOS SLN

Ex vivo study

Ex vivo permeability tests were important to assess the absorption-enhancing effects on intestinal tissue of a colloidal drug carrier device. The improvement in the SLN's permeability may also be induced by the villi's enterocyte, Peyer's cells, by a cellular pathway and trans cells. Table No 9 demonstrates SLN 's permeability up to one day. SLN has been consumed and reached into the lymphatic system. Observed ÍR 8.5 percent after 4 hours. SLN is intestinally permeable. Therefore it also seems from the findings that SLN are intestinal permeable.

Time (hours)	%CDR
0	0
1	3.7
2	5.7
4	8.5
18	19.05

Table 9 Ex-Vivo drug release Study

Stability study

Stability study of BOS-SLN dispersion and freeze dried BOS-SLN

There was no substantial improvement in MPS and percent EE as seen in Table 10. The medium size and DE percent were respectively 173.5 nm and 76.55 after 45 days. As a result, BOS SLN stable up to 45 days may be inferred.

Days	Particle Size (nm)	% Drug Entrapment (%)
0	**160.5**	**80.70**
30	**165.9**	**78.12**
45	**173.5**	**76.55**

Table 10 Stability study of dispersion of BOS SLN

Stability study of freeze dried BOS-SLN

As seen in Table 11, the mean particle size and proportion of drug content did not change considerably. After 45 days, average particle size and amount of drug trappings respectively were 182.5 nm and 75.12 percent. As a consequence, the freezing BOS SLN dried stable for up to 45 days may be inferred.

Days	Particle Size	% Drug content
0	**178.5**	**76.12**
30	**180.4**	**76.00**
45	**182.5**	**75.12**

Table 11 Stability study of freeze dried BOS SLN

CONCLUSION

During the present study, the planning, characterization and usage of hot homogenisation-ultra-sonication process for BOS loaded solid lipid nanoparticles was investigated. The sonic times, HSH rotation, HSH speed is configured, respectively, 2 min, RPM 15000 and 10 min. The drug: lipid (1:13) and surfactant (1 percent) concentration were designed with 32 complete factorial configuration. MPS 160.2±5.45 nm PDE 80.70±1.6% is designed for BOS SLN and −36.6 mw for surface charge. BOS, lipids and tailored formulations are also shown to be fully filled with the SLN by FTIR and DSC tests. We will assume from the TEM results that prepared SLNs are Spherical in form and smooth. It is also inferred from the description of the in-vitro and Ex-vivo drug launches that BOS SLN has released 94% CDR after 24 hours. Consequently, BOS-SLN preparation, FPM should be stopped because medication and lipid in nature are rather lipid. Also, Bosentan could become more bioavailable in order to digest them through the lymphatic route and circumvent FPM.

REFERENCES

1. Mullen, A., C. Schwarz, and W. Mehnert, (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. European journal of pharmaceutics and biopharmaceutics, 45(2): p. 149-155.
2. Schwarz, C., et al., (1994). Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. Journal of Controlled Release, 30(1): p. 83-96.
3. Mehnert, W. and K. Madder, (2001). Solid lipid nanoparticles: production, characterization and applications. Advanced drug delivery reviews, 47(2): p. 165-196.
4. Freitas, C. and R. Müller, (1999). Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. European journal of pharmaceutics and biopharmaceutics, 47(2): p. 125-132.
5. Manjunath, K. and V. Venkateswarlu, (2005). Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. Journal of Controlled Release, 107(2): p. 215-228.
6. Bakris, G.L., *et al.,* (1996). Calcium channel blockers versus other antihypertensive therapies on progression of NIDDM associated nephropathy. Kidney international, 50(5): p. 1641-1650.

7. Pucilowski, O., A. Płaźnik, and D.H. Overstreet, (1995). BOSadipine suppresses amphetamine-induced conditioned place preference and locomotor stimulation in the rat. Neuropsychopharmacology, 12(3): p. 239-244.
8. Argemí A., *et al*., (2011). Characterization of new topical ketoprofen formulations prepared by drug entrapment in solid lipid matrices. Journal of pharmaceutical sciences, 100(11): p. 4783-4789.
9. Sastry S.V., J.R. Nyshadham, and J.A. Fix, (2000). Recent technological advances in oral drug delivery–a review. Pharmaceutical science & technology today. 3(4): p. 138-145.
10. Videira M.A., et al., (2002). Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. Journal of drug targeting,. 10(8): p. 607-613.
11. Harde H., M. Das, and S. Jain, (2011). Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. Expert Opinion on Drug Delivery, 8(11): p. 1407-1424.
12. Bargoni A., et al.,(1998). Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. Pharmaceutical research, 15(5): p. 745-750.
13. Ekambaram, P., A.A.H. Sathali, and K. Priyanka, (2012). Solid lipid nanoparticles: A review. Sci Revs Chem Commun, 2(1): p. 80-102.
14. Patidar A., et al.,(2010). A review on novel lipid based nanocarriers. Int J Pharm Pharmaceut Sci, 4: p. 30-35.
15. lmeida A.J. and E. Souto, (2007).Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Advanced drug delivery reviews, 59(6): p. 478-490.
16. Freitas C. And R.H. Müller, (1998). Spray-drying of solid lipid nanoparticles (SLN< sup> TM</sup>). European journal of pharmaceutics and biopharmaceutics, 46(2): p. 145-151.
17. Garud A., D. Singh, and N. Garud,(2012). Solid Lipid Nanoparticles (SLN): Method, Characterization and Applications. International Current Pharmaceutical Journal, 1(11): p. 384-393.
18. Bunjes H., (2010). Lipid nanoparticles for the delivery of poorly water-soluble drugs. Journal of pharmacy and pharmacology, 62(11): p. 1637-1645.
19. Heurtault B., et al., (2003). The influence of lipid nanocapsule composition on their size distribution. European Journal of Pharmaceutical Sciences, 18(1): p. 55-61.
20. Jain P.,et al., (2009). Formulation development and characterization of solid lipid nanoparticles containing nimesulide. Int J Drug Deliv Technol, 1(1): p. 24-27.

ABBREVIATIONS

BOSadipine	BOS
Solid Lipid Nanoparticles	SLN
BOSadipine loaded Solid lipid nanoparticles	BOS-SLN
Biopharmaceutical Classification System	BCS
First Pass Metabolism	FPM
Mean Particle Size	MPS
% Entrapment Efficiency	%EE
%Cumulative Drug Release	%CDR
Transmission Electron Microscopy	TEM
Differential Scanning Calorimeter	DSC
Fourier transform infrared spectroscopy	FT-IR
Ultra Violet Spectrophotometer	UV-Spectrophotometer
Rotation Per Minute	RPM
Relative Humidity	RH
High Speed Homogenization	HSH
Analysis of variance	ANOVA