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Design, Characterization and In-Vitro Evaluation of Bosutinib Loaded Solid Lipid Nanoparticles

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Abstract

Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. Bosutinib (BST) is a BCS Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of BST leads to poor bioavailability, Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of BST. Animal data suggests that the absolute bioavailability of BST is about 14 to 34% due to an extensive first-pass effect. To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid–based drug delivery systems like solid lipid nanoparticles can be used. These systems enhance the lymphatic transport of the lipophilic drugs and therefore increase the bioavailability. Bosutinib can be conveniently loaded into solid lipid nanoparticles to improve the oral bioavailability by exploiting the intestinal lymphatic transport. The objective of this present investigation is enhancing the solubility and bioavailability of the drug Bosutinib by incorporating with different lipids such as dynasan 114, 116 and 118 by exploiting the intestinal lymphatic transport. The Bosutinib loaded solid lipid nanoparticles (BST-SLN) were prepared by hot homogenization followed by ultra-sonication method. The prepared SLN's were characterized for particle size, PDI, Zeta potential, EE, Total drug content and *in* vitro release study and its kinetic models.

Keywords: *Bosutinib, Solid Lipid Nanoparticles, Homogenization, Ultra sonication.*

Introduction

Oral drug delivery is the most preferable, convenient and widely used route of admin- istration for more than 90% of active pharmaceutical ingredients. It offers advan- tages like painless administration, no assis- tance and patient compliance as compared with other routes of administration, such as intramuscular, intravenous and pulmonary. However, several compounds are unsuccess- ful and failed owing to their low absorption and low bioavailability upon oral administra-tion [1].

The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action. Some of the reasons for poor bioavailability include: poor solubility; inappropriate partition coefficient as it influences the per- meation of drug through lipid membrane; first-pass metabolism; P-glycoprotein medi- ated efflux; and degradation of drug in the gastrointestinal tract (GIT) due to pH of the stomach or enzymatic degradation or by chemical [2]. Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. SLNs were developed in the last decade as an alternative system to the existing traditional carriers, i.e. emulsions, liposomes and polymeric nanoparticles [3, 4].These are related to emulsions, where the liquid lipid, oil is substituted by a solid lipid. SLNs offer unique properties such as small size, large surface area and high drug loadingand are attractive for their potential toimprove performance of active pharmaceutical ingredients (APIs). The advantages of SLNs include drug targeting, biocompatibility, nontoxicity, drug release modulation and small scale production [5].

The main objective of this present investigation is enhancing the solubility and bioavailability of the drug Bosutinib by incorporating with different lipids such as dynasan 114, 116 and 118 by exploiting the intestinal lymphatic transport. The Bosutinib loaded solid lipid nanoparticles (DS-SLN) were prepared by hot homogenization followed by ultra-sonication method. The prepared SLNs were characterized for particle size, PDI, Zeta potential and in vitro release study and its kinetics.

Materials

Bosutinib were obtained as gift sample from Aurobindo labs, Hyderabad. Dynasan 114, 116, 118 were purchased from M/s Sigma – Aldrich Chemicals, Hyderabad. Soy Lecithin E-80 was a gift sample from M/s. Lipoid, Germany. Methanol, acetonitrile, chloroform and Dichloromethane were of HPLC grade (Merck, India). Centrisart filters (molecular weight cut off 20,000) were purchased from M/s. Sartorius, Goettingen, Germany.

Methods

Preparation of BS-SLNs and Drug Suspension

Bosutinib loaded solid lipid nanoparticles were prepared by hot homogenization followed by the ultrasonication method [6]. Table 1 shows the composition of BS-SLNs.In 10 mL of 1:1 mixture of methanol and chloroform, drug Bosutinib, solid lipid (Dynasan 114,116 & 118) and emulsifier soy lecithin were dissolved to get oil phase. The aqueous phase was prepared with poloxamer 188 by dissolving in distilled water to get 0.5 to 2 %w/v solution. Organic solvents were completely removed by a rotary flashevaporator. The drug embedded lipid layer was molten by heating to 5ºC above the melting point of the lipid to get a clear homogenous lipid phase. The aqueous phase was heated to the same temperature as that of the lipid phase.

To the hot homogenous lipid phase, the hot aqueous surfactant solution was added and homogenized at 12,000 rpm, for 4 min, with DIAX 900 high-speed homogenizer (Heidolph, Germany). The obtained coarse oil in water emulsion was subjected to sonication using a probe soincator (Vibracell, USA; 12T-probe) for 20 min. To prevent the precipitation during homogenization and ultrasonication, the temperature was maintained at least 5ºC above the lipid melting point.

Bosutinib loaded SLNs were finally obtained by allowing the hot nanoemulsion to cool to room temperature. A coarse suspension of Bosutinib was prepared by adding 10 mg of Bosutinib to 50 mg of sodium carboxy methyl cellulose in a mortar and triturated together for 3 min. Further, 10 mL of distilled water was added and triturated for 5 min to get the suspension of DS.

Table 1: Composition of Bosutinib Loaded solid lipid nanoparticles and suspension

Formulation ingredients	F1	F2	F3	F4	F5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	BS SUS
Bosutinib (mg)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Dynasan-114 (mg)	100	200	300	200	200	-	-	-	-	-	-	-	-	-	-	-
Dynasan-116 (mg)	-	-	-	-	-	100	200	300	200	200	-	-	-	-	-	-
Dynasan-118 (mg)	-	-	-	-	-	-	-	-	-	-	100	200	300	200	200	-
Soylecithin95%(mg)	50	100	150	100	100	50	100	150	100	100	50	100	150	100	100	-
Polaxomer 188 (%)	0.5	1.0	1.5	1.75	2.0	0.5	1.0	1.5	1.75	2.0	0.5	1.0	1.5	1.75	2.0	-
Double distilled water(mL)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Chloroform : Methanol (1:1)(mL)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-
Sodium CMC (mg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50

Characterization of SLNs

Determination of Particle Size, PDI andZeta potential of SLNs

Mean diameter of the formulation and polydispersity index (PDI) were determined by photon correlation spectroscopy (PCS)

using a Zetasizer Nano ZS90 (Malvern Instruments, UK).

The prepared solid lipid nanoparticle preparations were diluted in 1:50 ratio with double distilled water and size was measured at 90ºangles [7]. All measurements were done in triplicate. The surface charge was determined by measuring the zeta potential (ZP) of SLN based on the Smoluchowski equation and ZP measurements were made at 25º C.

Measurement of Entrapment Efficiency

To determine the entrapment efficiency (EE), about 2.5 mL of SLN formulation was subjected to centrifugation using Centrisart separators (Sartorius, Germany) at 4000 rpm for 15 min, which had a separating membrane with 20,000 Da molecular weight cut off to separate the ultra filtrate [8]. This ultra filtrate contained the un-entrapped drug, which was estimated by high performance liquid chromatography (HPLC) method [9]. The concentration of free drug in aqueous medium was estimated and the EE was calculated.

Determination of Total Drug Content

About 0.1 mL of the SLN formulation wasdissolved in 0. 9 mL of a 1:1 mixture of chloroform and methanol and then further diluted with mobile phase [10]. The drug in diluted samples was estimated by HPLC.

In Vitro Drug Release Studies

In vitro release studies of DS-SLN formulations were done in 0.1 N HCl (pH 1.2) for 2 h followed by pH 6.8 phosphate buffer for 22 h, by using dialysis technique [11]. During in vitro release studies, dialysis membrane (Himedia, India) with a molecular weight cut-off ranging from 12,000 to 14,000 Da was used. The dialysis membrane was kept in double distilled water for overnight. The apparatus for release study had a donor and a receptor compartment.About 2 mL of SLN dispersion was taken for release study in donor compartment, which consisted of a boiling tube with opening at one end and tied with dialysis membrane at the other end. A 250 mL beaker was used as receptor compartment with 100 mL release medium and the temperature was maintained at 37 ± 0.5ºC. About 2 mL samples were withdrawn from receiver Compartment and were replaced with fresh medium periodically at intervals of 0.5, 1, 2 h in 0.1 N HCl medium and followed by 2, 4, 8, 12, 16, 20 and 24 h in pH 6.8 phosphate buffer medium. The collected samples were suitably diluted and analyzed by UV Visible Spectrophotometer at λ_{max} 321nm [12].

In Vitro Release Kinetics from DT-SLN

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of Bosutinib release from SLN's. The kinetic models used were zero order equation, first order equation, Higuchi release and Korsemayer-peppas equation.

Stability Studies

DS loaded SLNs were stored for stability testing at room temperature and refrigerated temperature for Six months. The stored samples of SLNs were checked at monthlyintervals for particle size, PDI, ZP, EE and total drug content for six months.

Lyophilization of SLNs

Lyophilization was done to enhance the stability of the SLN preparation. The SLN's containing 10% w/v mannitol were prepared and kept in deep freezer at -40 º C (Sanyo, Japan) for overnight. The frozen samples were then transferred into freeze-dryer (Lyodel, Delvac Pumps Pvt. Ltd, India) [13, 14]. The periods for preliminary freezing, freeze drying, applied vacuum andtemperature conditions were optimized.

The obtained freeze-dried SLN free flowing powder was stored and used for further studies i.e., solid state characterization (DSC & XRD) and surface morphology by SEM

Solid State Characterization Differential Scanning Calorimetry

DSC is one of the basic tools used to investigate drug-excipient compatibility and crystalline behavior of drugs and other excipients. DSC thermograms of pure drug, pure lipids (Dynasan-114, 116 and 118), and their physical mixtures (PM in 1:1 ratio) and optimized SLN formulation were obtained by Perkin Elmer (DSC 4000, USA) instrument in the range of 60–200ºC with 10ºC/min heating rate. Nitrogen was used as purging gas. DSC instrument was calibrated by using indium. Standard aluminum pans were used containing 10 mg of samples [15].

Powder X-ray Diffractometry (PXRD)

The X-ray diffraction studies were conductedby using powder X-ray diffractometer (XRD 6000, Shimazdu, Japan). In this technique, the samples were exposed to nickel filtered CuKa radiation (40 kV, 30 mA) and scanned from 2º to 70º, 2Θ at a step size of 0.045º and step time of 0. 5 s. Samples used for PXRD analysis were pure Bosutinib, Dynasan-118, physical mixtures of drug withdynasan- 118 (1:1 ratio) and lyophilized optimized DS-SLN.

Morphology Study by Scanning ElectronMicroscopy (SEM)

The morphology of BS-SLNs was studied by Scanning Electron Microscopy (SEM) (S- 4100, Hitachi, Japan). Initially, the freeze dried SLN formulation was fixed on to the carbon coated brass stub. This was sputter coated with Platinum coating machine (JEOL, JFC-1600 Auto fine coater) and mounted in SEM (JSM- 6510LA, JEOL, Japan) for surface analysis by applying 15,000 V at various magnifications [16].

Results and Discussion

Optimization of Process Parameters andVariables

Bosutinib loaded solid lipid nanoparticles were prepared by hot homogenization followed by ultra-sonication is a reliable, simple and reproducible method for preparing SLN. Initially,homogenization ofthe lipid phase with hot aqueous poloxamer solution for 10 min was used to produce a coarse emulsion followed by sonication resulting in average particle size between 190 and 250 nm. A further increase in homogenization time showed increase in particle size to a greater extent. Thus, 10 min homogenization time wasselected for all the formulations and further reduction of size was attained with sonication. Sonicating the coarse emulsion for 20 min resultedin particles between 170 and 230 nm with narrow size distribution. Inorder to optimize the lipidto drug ratio, different amounts of all three lipids (100, 200 and 300 mg) were tried with fixed

amount of drug (10 mg). Lipid content 200 mg of dynasan 114,116 and 118, helped in the good encapsulation efficiency (Table 2). Based on these results, the lipid to drug ratio of 200:10 for dynasan 114, 116 and 118 were used for further studies.
To obtain stable and smaller SLNs, poloxamer concentration was varied from 0.5% to 2% and their effect on particle size was measured. A poloxamer concentration of 1.75% was effective in producing smaller size SLN, further increase in concentration leads to greater particle size.

These results clearly suggested that an optimum concentration of 1.5% poloxamer was sufficient to get nanoparticles effectively and prevented agglomeration during the homogenization process. High concentration of surfactant (2%) was avoided to prevent decrease in the entrapment efficiency andalso toxic effects associated with surfactants (Müller et al., 2000).

Measurement of Particle Size, PDI and Zeta Potential

All the formulations were analyzed in order to determine their particle size distribution, PDI and zeta potential values. The results are represented in Table 2. The particle size of all the formulations ranged from 150.73±4.47 nm to 330.29±5.79 nm, PDI from 00.20±0.097 to 0.44±0.028 and zeta potential from -15.9±1.87 to -29.1±2.16 mV.

From the results obtained, formulations containing Dynasan-114 showed lower particle sizes but the PDI was higher and zeta potential was lower. Formulations containing Dynasan-116 showed higher particle sizes but PDI and zeta potential was lower. But formulations containing Dynasan-118 showed good particle size, lower PDI andhigher zeta potential, when compared to other formulations.

Determination of Entrapment Efficiencyand Total Drug Content

All the formulations were analyzed for entrapment efficiency and total drug content by HPLC. Results are tabulated and represented in Table 2. From the results obtained, all formulations showed good entrapment efficiency ranging from 72.47±0.21 % to 93.56±0.21 % and total drug content ranging from 9.02±0.04 to 9.60±0.02 mg.

Formulation containing Dynasan-118 showed higher values of entrapment efficiency of 93.56% and total drug content of 9.66 mgwhen compared to Dynasan-114 and Dynasan-116.(As represented in Table No.2).

Table 2: Determination of Particle Size, PDI, ZP, EE and Total drug Content

Formulation code	Size (nm)±SD	PDI ± SD	Zeta potential (mV) ± SD	Entrapment efficiency (%)	Total drug content (mg)
F1	160.23±5.96	0.44±0.028	-15.9±1.87	72.47±0.21	9.09±0.05
F2	172.52±7.52	0.37±0.035	-18.7±1.78	75.82±0.11	9.13±0.09
F3	230.95±3.45	0.36±0.013	-20.2±1.01	82.71±0.31	9.37±0.02
F4	**167.80±5.87**	**0.25±0.016**	**-21.9±1.87**	**89.23±0.10**	**9.52±0.01**
F5	192.23±2.89	0.39±0.012	-21.7±1.78	86.08±0.24	9.16±0.07
S1	200.42±3.12	0.35±0.018	-18.2±1.34	84.35±0.42	9.02±0.04
S2	260.52±4.25	0.31±0.021	-23.4±1.31	87.58±0.21	9.23±0.01
S3	318.28±6.28	0.27±0.017	-20.1±1.72	89.47±0.31	9.47±0.02
S4	**245.30±4.92**	**0.21±0.031**	**-25.9±1.56**	**91.38±0.21**	**9.54±0.07**
S5	271.76±8.52	0.28±0.084	-23.7±1.45	89.26±0.25	9.40±0.03
E1	220.23±5.26	0.26±0.028	-22.1±1.58	86.36±0.32	9.03±0.05
E2	290.52±2.52	0.21±0.035	-25.3±1.25	90.28±0.41	9.27±0.03
E3	330.25±3.45	0.27±0.013	-23.2±1.01	91.75±0.25	9.39±0.08
E4	**150.73±4.47**	**0.20±0.097**	**-29.1±2.16**	**93.56±0.30**	**9.60±0.02**
E5	300.29±5.79	0.23±0.015	-26.3±2.06	90.06±0.09	9.23±0.01

Drug Release from SLNs by DialysisMethod

Formulations containing Dynasan-114, Dynasan-116 and Dynasan-118 (F4, S4, E4) showed a maximum drug release of 68.79%,73.66%, 81.55% respectively in 0.1N HCl followed by pH 6.8

phosphate buffer. E4 formulation showed highest percentage drug release among all the prepared SLNs. All the experiments were carried out in triplicate.

Table 3: *In vitro* percentage drug release from Bosutinib loaded SLN formulations in pH 1.2 and pH 6.8 buffer (Mean± SD, n=3)

Time(h)	**F4**	**S4**	**E4**
0	0	**0**	**0**
1	16.60±1.11	17.90±1.03	21.12±1.65
2	21.18±1.03	28.78±1.01	30.28±1.22
4	27.72±1.04	36.42±1.21	35.67±1.13
8	35.71±1.29	45.71±1.13	47.23±1.25
12	43.88±0.80	54.38±1.00	56.77±0.56
16	50.98±1.25	61.58±1.09	65.46±1.89
20	60.67±1.25	69.95±1.10	73.98±1.55
24	68.79±1.05	73.66±1.21	80.55±1.10

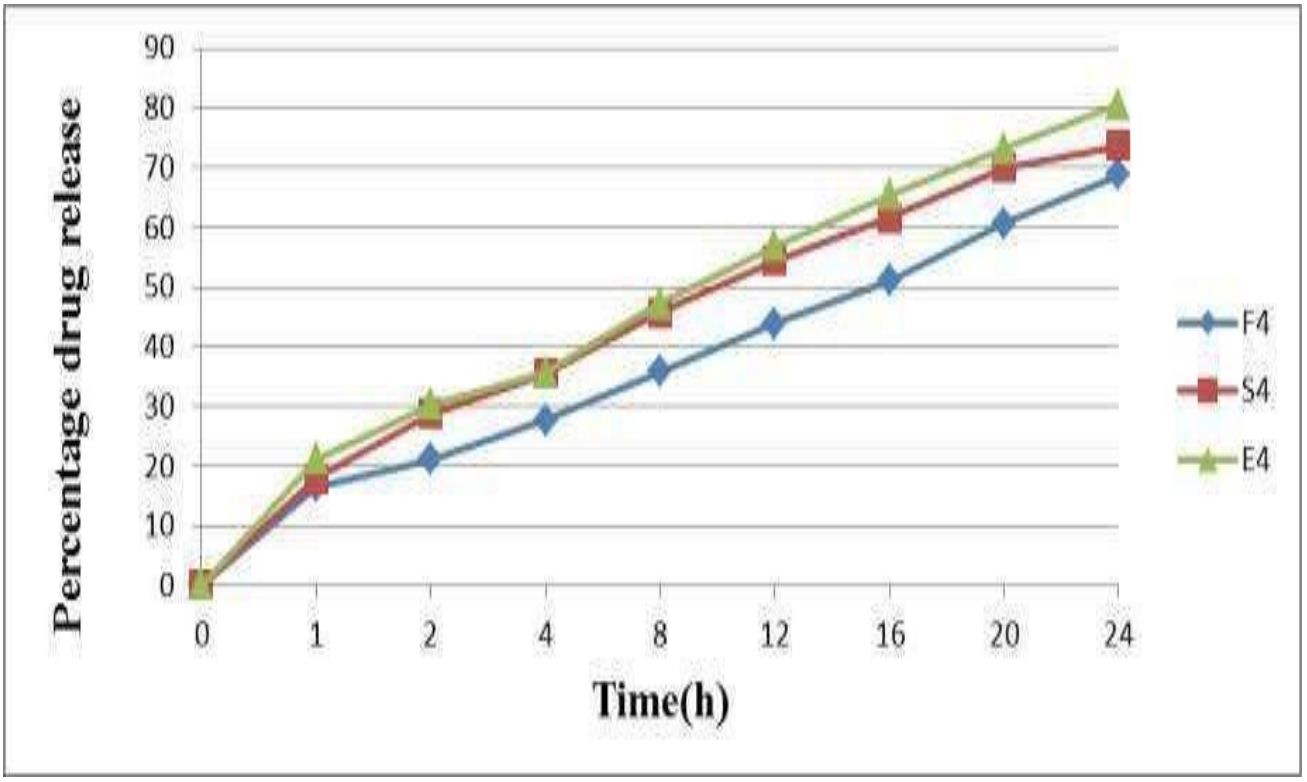


Figure 1: Comparison of *in vitro* drug release from Bosutinib loaded SLN with different lipid formulations (Dynasan 114, 116 and 118) F4, S4, E4 through dialysis membrane

In Vitro Release Kinetics

The obtained release equations and regression coefficients (R^2**)** for BST-SLN enriched with SLN are shown in Table.R elease of drug from BST-SLN (F4, S4, and E4) followed Higuchi, the best fit with the highest correlation coefficients were shown in Higuchi plot.

The mechanism of release is by diffusion as indicated by R^2 value of Higuchi and n value of Korsemeyer-Peppas equation.

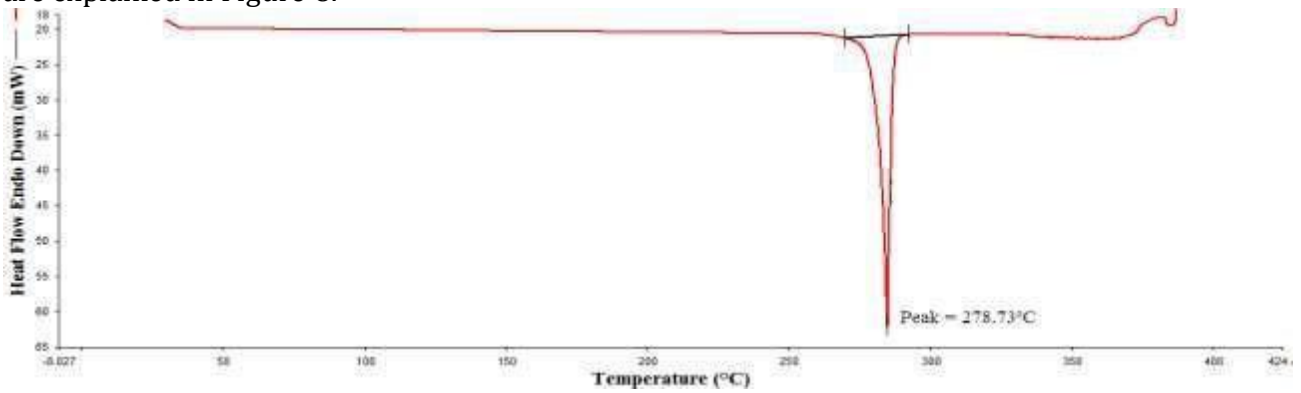
The percentage drug release was proportional to square root of time (**Higuchi Model**) indicating that drug release from SLN enriched with SLN is diffusion Controlled. More over the plots of log percentage release vs. log time (Peppas) showed a high level linearity as given in table, which in turn another conformationthat the release is diffusion controlled.

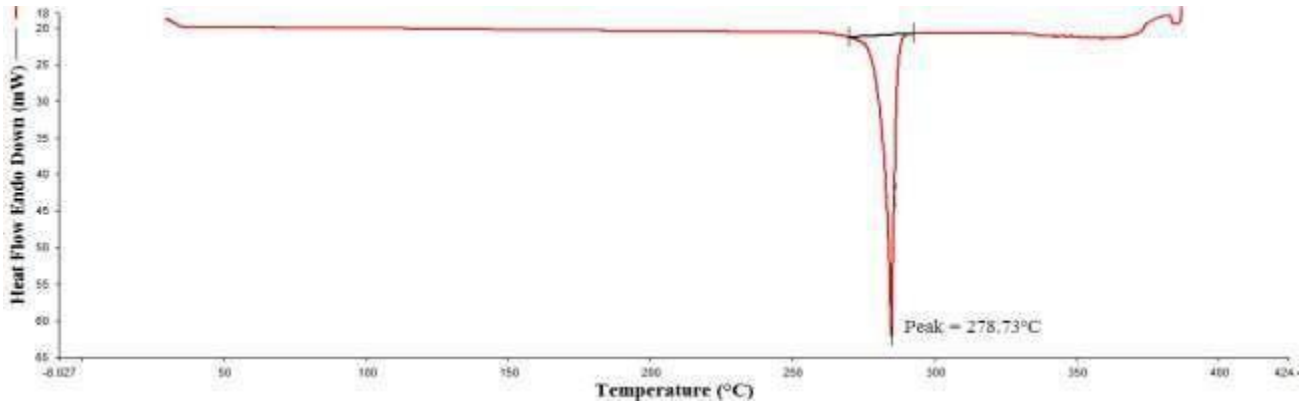
Table 4: Regression coefficient (R^2) values of Bosutinib loaded SLN through dialysis membrane

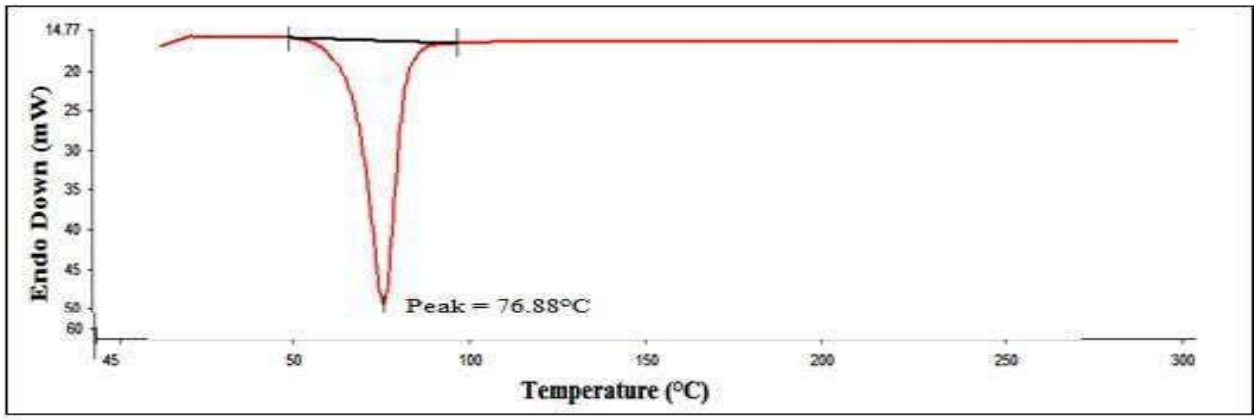
Formulations	**R^2 value**				**n** value
	Zero-order	**First order**	**Higuchi**	**Korsmeyer - Peppas**	
F4	0.942	0.977	**0.989**	0.985	0.437
S4	0.887	0.972	**0.991**	0.987	0.422
E4	0.921	0.982	**0.992**	0.988	**0.405**

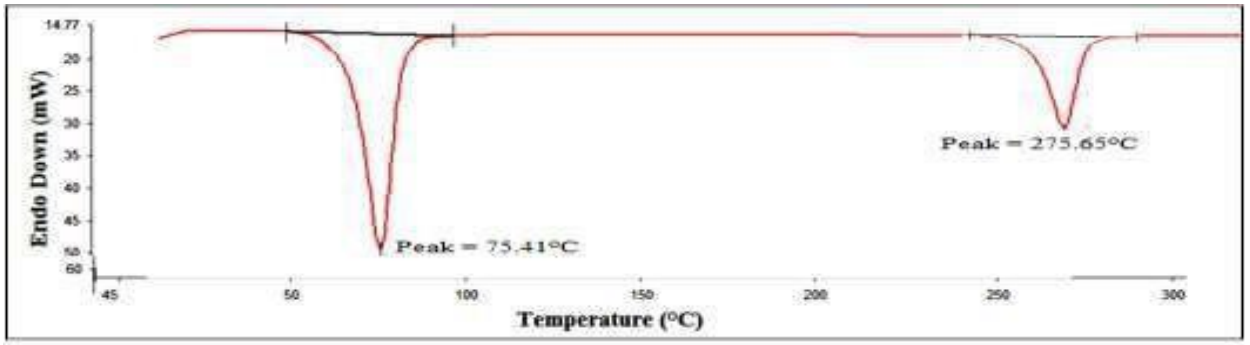
Solid State CharacterizationDSC

Differential Scanning Calorimeter was used to study the thermal analysis of drug- excipient compatibility. Pure DS, Pure lipid(Dynasan-118), Physical mixture of drug and lipid (1:1) and freeze dried SLN preparationwere scanned in the temperature range of 50- 300˚C. The results of DSC are explained in Figure 8.









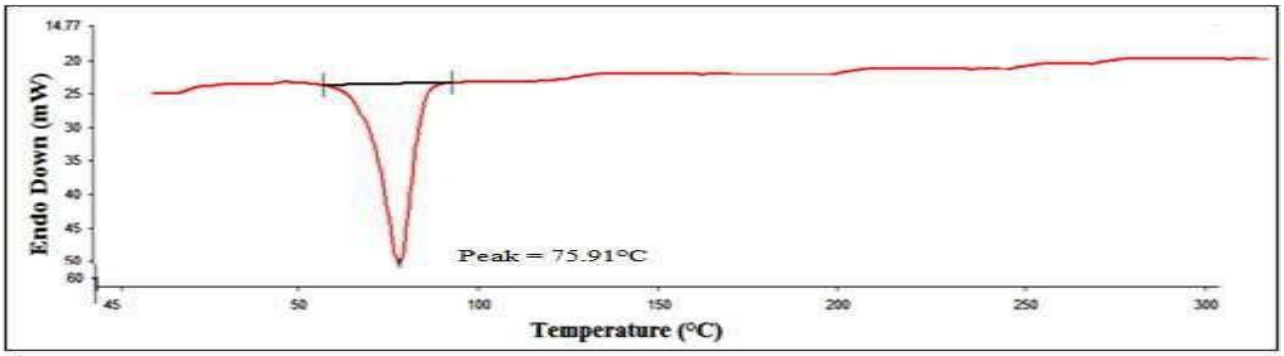


Figure 2: DSC thermograms of Pure DS, Pure Dynasan-118, Physical mixture and lyophilized (E4) SLN formulation

From the results, pure BS showed sharp endothermic peak at 278.73°C. In case of pure lipid, a sharp melting endotherm peak was observed at 76.88°C. The drug peak was preserved at 275.65°C in physical mixture but, absent in lyophilized SLN formulation. This indicates the conversion of drug from crystalline to amorphous form.

X-ray Diffraction Studies

Overlay XRD spectrum of DS, Pure lipid Dynasan-118, Physical mixture of BS and Dynasan-118 and lyophilized (E4) SLN was showed in Figure 3. The XRD spectrum of DS displayed sharp peaks at 2-theta angles of20.15, 21.27, 21.61, 24.20, 24.83, 26.89, 28.24,
29.37 and numerous minor peaks up to 30. The peaks that were observed were well resolved and intense, demonstrating the crystalline nature of DS. The diffraction patterns of the Dynasan-118 showed various minute diffuse peaks. The XRD spectrum of the lyophilized SLN exhibited broad diffuse peaks resulting in the decrease in the crystalline nature of the DS, suggesting that BS had been converted into amorphous nature.

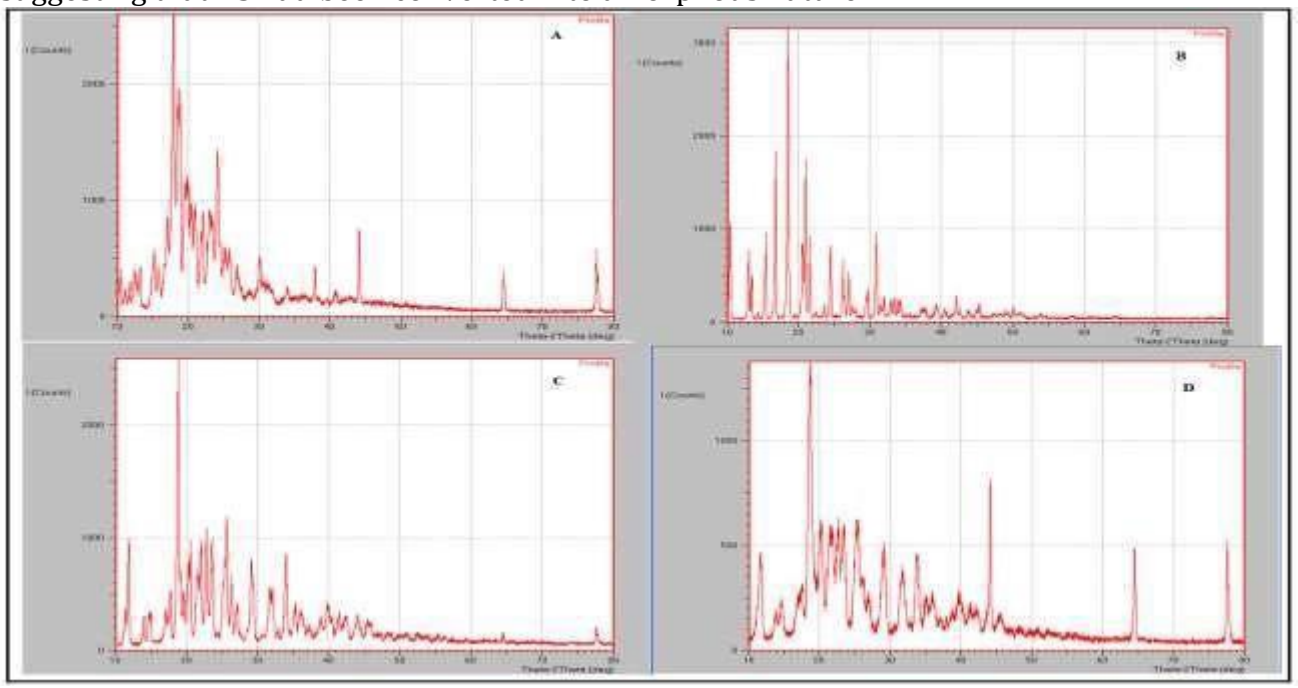


Figure 3: PXRD Spectra's of pure B S (A), Pure Dynasan-118 (B), Physical mixture (C) and Lyophilized (E4) SLN formulation (D)

Study of DS-SLN Morphology byScanning Electron Microscopy (SEM)

SEM studies of BST-SLN were carried outand showed in Figure 4.
The particles are spherical in shape withincreased polydispersity due to aggregation.

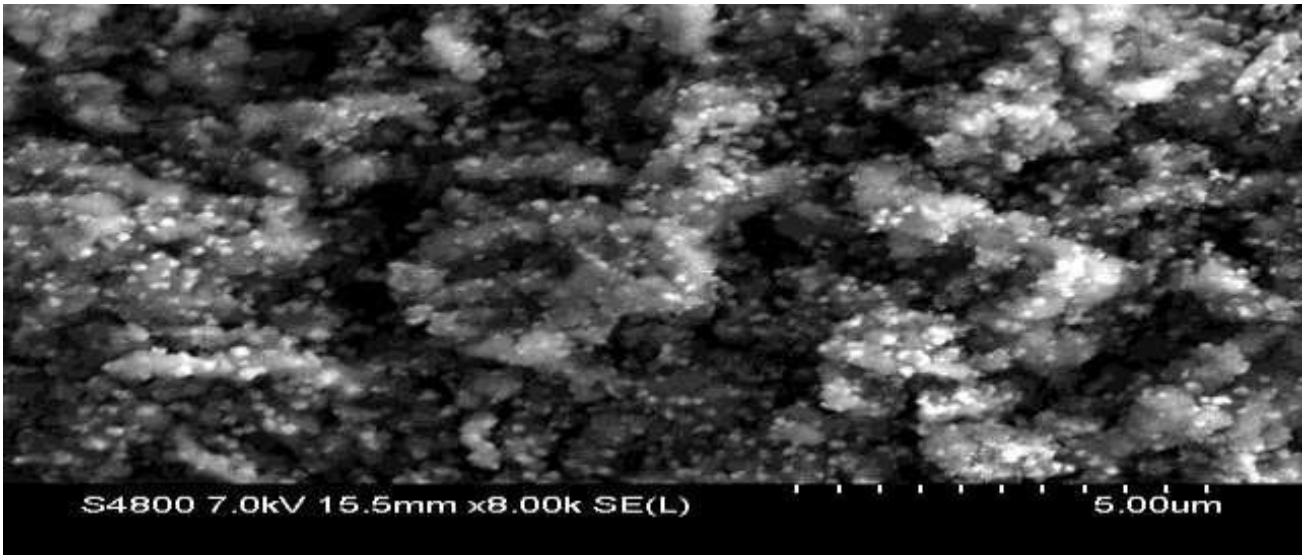


Figure 4: SEM image of DS-SLN (E4) formulation

Physical Stability Studies

Bosutinib loaded solid lipid nanoparticles of optimized formulation (E4) were stored at room and refrigerated temperature for 180 days, and average size, zeta potential and poly dispersity index, EE and Total drug content were determined. The samples were estimated in triplicate.

Some changes were noticed in size, PDI and zeta potential values, which indicated the susceptibility for stability problems duringstorage at room temperature and 4^{o}C.

Hence, lyophilization technique was used to overcome this problem. Collected datas were subjected to statistical significance with t- test using Graph pad prism software (version 5. 02.2013) at the probability level of $p < 0.05$.

Conclusion

Bosutinib loaded Solid Lipid Nanoparticles were prepared by hot homogenization followed by ultra-sonication method.Formulations containing Dynasan-118(E4)showed good particle size, lower PDI, higher zeta potential, higher values of entrapment efficiency of 93.56% and total drug content of9.66mg when compared to Dynasan-114 andDynasan-116.

E4 formulation showed highest percentagedrug release among all the prepared SLNs. Release of drug from BST-SLN (F4, S4, and E4) followed Higuchi, the best fit with thehighest correlation coefficients were shown in Higuchi plot. The mechanism of release is by diffusion as indicated by R^2 value of Higuchi and n value of Korsemeyer- Peppas equation. Physical stability study datas are statistically significant at a level of $p<0.05$.

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