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STUDIES IN DEVELOPMENT OF DASATINIB NANOFORMULATIONS

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ABSTRACT

Dasatinib (DST) is an anti-cancer drug having poor water solubility and high permeability. Low aqueous solubility and poor dissolution of DST results into poor and variable bioavailability. Its limited aqueous solubility and high lipophilicity limits the therapeutic outcome. The aim of present work was to increase solubility of DST which may increase the bioavailability. Nanoformulations of DST such as Nanoparticle, Solid Lipid Nanoparticle (SLNs) and Nanocrystals were prepared and evaluated for physicochemical characteristics. Finalised Nanoformulations were compared with respect to Solubility, *In-Vitro* drug release, *In-Vitro* cytotoxicity and Bioavailability study. From the physicochemical characterization and evaluation of all nanoformulations, it was observed that Polylactic-co-glycolic acid (PLGA) Nanoparticles prepared using solvent-anti solvent precipitation method has higher increment in the solubility compare to SLNs and Nanocrystal of DST. Cytotoxicity Study showed that PLGA Nanoparticles have higher bioavailability as well as higher % Cell inhibition. In a nutshell, Nanoformulation such as PLGA Nanoparticle is one of the promising approach to enhance the Bioavailability of DST.

KEYWORDS: Dasatinib (DST), Nanoparticles, Solid Lipid Nanoparticles (SLNs), Nanocrystals, Bioavailability, Poly lactic-co-glycolic acid (PLGA).

1. INTRODUCTION

Formulation of poorly water soluble molecule is one of the most difficult and challenging tasks for the formulation scientist . An enhancement in the solubility and dissolution rate can improve the oral bioavailability of such molecules which further improves the therapeutic efficacy as well. Dasatinib (DST) is a BCS Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of DST leads to poor bioavailability. Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of DST. Animal data suggests that the absolute bioavailability of DST is about 14 to 34% due to an extensive first-pass effect.

Polylactic-co-glycolic acid (PLGA) is one of the most successfully developed biodegradable polymer among the different polymers developed to formulate polymeric nanoparticles. It gives sustained release as well as increase solubility of drug.[1, 2] SLNs consist of a solid lipid matrix where the drug is incorporated. Nanocrystals are aggregates of molecules that combine in a crystalline form which contains pure drug and surfactant. To avoid aggregation and to stabilize the SLNs and Nanocrystal, surfactants are used in the present research work which has an accepted GRAS (Generally Recognized as Safe) status.[3]

2. MATERIALS AND METHODS

2.1 MATERIALS

Dasatinib and PLGA were kindly supplied by Sun Pharma, Baroda, India as a gift sample. Tween 80 and Glyceryl monostearate were obtained as a gift sample from Croda and Gattefosse respectively. Poloxamer 188 was supplied by Sigma–Aldrich, Bangalore, India. Triton X100 was supplied by S D Fine Chem Ltd., Mumbai, India as a gift sample. All remaining reagents and chemicals were of analytical grade. Purified water used for all experiments was MilliQ Plus, Millipore.

2.2 ANALYTICAL METHOD DEVELOPMENT USING UV TECHNIQUE

Accurately weigh 10 mg of DST and transfer it into 100 ml of volumetric flask. Add DMSO and make up the volume up to mark (100 µg/ml). Withdraw the aliquots of 0.2 to 0.9 ml and dilute it up to 10 ml of distilled water (2 to 9 µg/ml). Measure the absorbance of solutions using UV spectrometer (Shimadzu 1800, Japan) at 326 nm. Plot the graph of absorbance vs. concentration.[4]

Table 1: UV Calibration Curve Data in DMSO.

Concentration (µg/ml)	Absorbance				SD (±)
	1	2	3	Average	
2	0.190	0.179	0.187	0.185	0.03
3	0.278	0.274	0.277	0.276	0.02
4	0.363	0.364	0.372	0.366	0.04
5	0.451	0.458	0.462	0.457	0.03
6	0.575	0.580	0.593	0.582	0.01
7	0.676	0.676	0.691	0.681	0.02
8	0.773	0.776	0.793	0.780	0.04
9	0.847	0.861	0.877	0.861	0.03
Y= 0.0991X-0.0213				**R^2= 0.9981**	

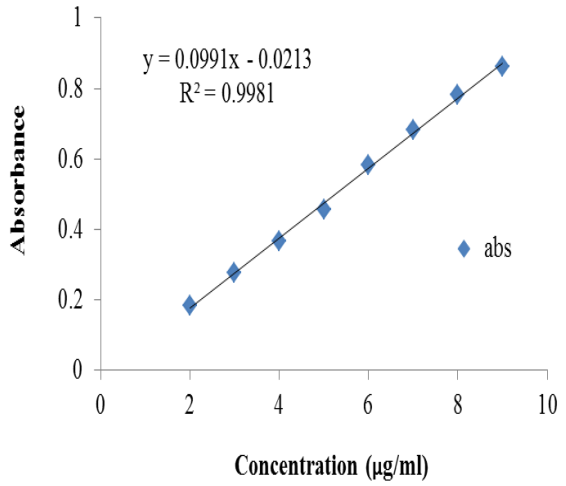

Figure 1: UV Calibration Curve at 326 nm (Absorbance vs. Concentration)

Calibration Curve of DST in pH 4 Acetate buffer by UV Spectroscopy

Accurately weigh 10 mg of DST and transfer it into 100 ml of volumetric flask Add pH 4 Acetate buffer and make up the volume up to mark with same (100 µg/ml). Withdraw the aliquots of 0.2 to 1.0 ml and dilute it up to 10 ml of distilled water (2 to 10 µg/ml). Measure the absorbance at 320 nm using UV spectrometer Shimadzu 1800, Japan. Plot the graph of absorbance v/s concentration.

Table 2: UV Calibration Curve Data in pH 4 Acetate Buffer.

Concentration (µg/ml)	Absorbance				
	1	2	3	Average	
2	0.115	0.117	0.119	0.117	0.03
4	0.224	0.221	0.222	0.222	0.02
6	0.357	0.355	0.359	0.357	0.03
8	0.478	0.477	0.479	0.477	0.04
10	0.631	0.629	0.630	0.630	0.01
Y= 0.0643X-0.0248	**R^2= 0.996**				

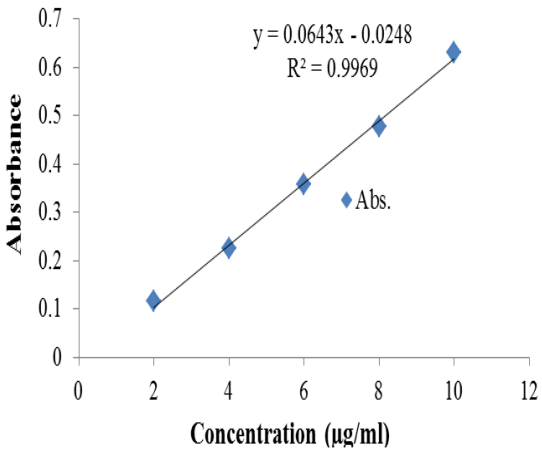

Figure 2: UV Calibration Curve at 320 nm (Absorbance vs. Concentration)

2.3 FORMULATION DEVELOPMENT OF PLGA NANOPARTICLES / SLNS / NANOCRYSTALS

Nanoparticles were prepared by precipitation and solvent evaporation method. Briefly, accurately weighed DST was dissolved in 7 ml ethanol and PLGA was dissolved in 3 ml of acetoneto form organic solution. Specified quantities of stabilizers were dissolved in 25 ml water (anti-solvent system). Both solutions were passed through a 0.45µm filter (Gelman Laboratory, Mumbai, India). The anti-solvent was kept below 8°C in an ice-water bath. Then, organic solution was added drop by drop with the use of syringe in a continuous (A) Homogenization condition under high speed homogenizer. Initially a nanoparticle was characterized by bluish tint of the resulting solution after homogenization. Homogenization speed of 15000 RPM was maintained for 30 minutes followed by sonication for 10minutes. (B) Using High Pressure Homogenization at 10,000 PSI Pressure for 10 cycles. All process parameters were optimized and found to be same as mentioned in above process. Nanoparticles containing ampoules with the addition of D-mannitol (5%w/v) as cryoprotectant were frozen in deep freezer at -95°C for 8h for primary freezing. The ampoules were then transferred to flask and the flask was attached to the vacuum adapter of lyophilizer. Based on the visual inspection of Lyophilized product like quality and quantity, the Lyophilization cycle was carried out under vacuum (pressure of 600mTorr for 2 hr, then at 400 mTorr for 10 hr. then at 250 mTorr for 10 hr& 150 mTorr for 2 hr for 24 h at +20°c temperature Ampoules and -98°c condenser temperature.

Solid Lipid Nanoparticles (SLNs) were prepared by precipitation and solvent evaporation method. Briefly, accurately weighed DST was dissolved in 7 ml ethanol to form organic solution of drug. Specified quantities of stabilizers were dissolved in 25ml water (anti-solvent system). Both solutions were passed through a 0.45µm filter (Gelman Laboratory, Mumbai, India). The anti-solvent was kept below 8 °C in an ice-water bath. Then,

organic solution was added drop by drop with the use of syringe in a continuous (A) Homogenization condition under high speed homogenizer. Initially Solid Lipid Nanoparticles (SLNs) was characterized by bluish tint of the resulting solution after homogenization. Homogenization speed of 10000 RPM was maintained for 30minutes followed by sonication for 10 minutes. (B) Using High Pressure Homogenization at 10000 Psi Pressure for 10 cycles. Solid Lipid Nanoparticles (SLNs)s containing ampoules with the addition of D-mannitol (5%w/v) as cryoprotectant were frozen in deep freezer at -95°C for 8h for primary freezing. The ampoules were then transferred to flask and the flask was attached to the vacuum adapter of lyophilizer. Based on the visual inspection of Lyophilized product like quality and quantity, the Lyophilization cycle was carried out under vacuum.

Nanocrystals were prepared by precipitation and solvent evaporation method. Briefly, accurately weighed DST was dissolved in 7 ml ethanol to form organic solution of drug. Specified quantities of stabilizers were dissolved in 25ml water (anti-solvent system). Both solutions were passed through a 0.45µm filter (Gelman Laboratory, Mumbai, India). The anti-solvent was kept below 8 °C in an ice-water bath. Then, organic solution was added drop by drop with the use of syringe in a continuous (A) Homogenization condition under high speed homogenizer (Kinematica, polytron. Pt 2500E). Initially Solid Lipid Nanoparticles (Nanocrystals)s was characterized by bluish tint of the resulting solution after homogenization. Homogenization speed of 15000 RPM was maintained for 30 minutes followed by sonication for 10 minutes. (B) Using High Pressure Homogenization at 10000 PSI Pressure for 10 cycles. Nanocrystals containing ampoules with the addition of D-mannitol (5%w/v) as cryoprotectant were frozen in deep freezer at -98°C for 1h for primary freezing. The ampoules were then transferred to flask and the flask was attached to the vacuum adapter of lyophilizer.

2.4 OPTIMIZATION OF DST PLGA NANOPARTICLES/ SLNS / NANOCRYSTALS

2.4.1 Preliminary batches for selection of surfactant

Tween 80 and Poloxamer are generally use as a surfactant in nano particles preparation. From the literature, it was seen that both surfactants are generally use in the range of 1-2 % w/v. Six batches were prepared with two surfactants in different concentration. The evaluation of surfactant was done using parameter like precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.4.2 Preliminary batches for selection of homogenization speed

Literature suggests that homogenization speed, generally 10000-20000 rpm is sufficient for nano particle preparation. Three batches were prepared at 10000, 15000 and 20000 rpm. The evaluation of homogenization speed was done using parameter precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.4.3 Preliminary batches for selection of homogenization time

Literature suggest that 15-45 min homogenization time is sufficient to prepare nano particle. Three batches were prepared and the evaluation of homogenization time was done using parameter like precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.4.4 Preliminary batches for selection of Pressure using High Pressure Homogenizer (HPH) (Avestine)

Literature suggests that 5000-15000 psi pressure is sufficient to prepare nano particles. Three batches were prepared and the evaluation of Pressure was done using parameter like precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.4.5 3^2 factorial design layout

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time by trial and error method which is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as experimental design.

In addition to the art of the formulations, the technique of factorial design is effective method of indicating the relative significance of a number of variables and their interactions. The number of experiment required for these studies is dependent on the number of independent variable selected. The response (Y_i) is measured for each trial.

$$Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_1^2+b_{22}X_2^2$$

Where Y is dependent variable, b_0 is the arithmetic mean response of the nine runs and b_1 is estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at time from its low to high values. The interaction term (X1X2) shows how the response changes when two factors are simultaneously changed. The polynomial term (X_1X_1 and X_2X_2) are included to investigate non linearity. The polynomial equation can be used to draw conclusion after considering the magnitude of coefficient and the mathematical sign it carries. (I.e. positive or negative)

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The design layout

and coded value of independent factor is shown in table, respectively. The %w/v of surfactant tween 80 and Drug: polymer ratio medium were selected as independent variables. The % particle size was selected as dependent variable. The formulations of the factorial batches (F_1 to F_9) are shown in table.

Table 3: 3^2 factorial design layout for PLGA Nanoparticles

Batch code	X_1	X_2
P1	-1	-1
P2	-1	0
P3	-1	1
P4	0	-1
P5	0	0
P6	0	1
P7	1	-1
P8	1	0
P9	1	1

Where, X_1 code for tween 80 w/v and X_2 code for drug: PLGA ratio.

Table 4: 3^2 factorial design layout for SLNs

Batch code	X_1	X_2
S1	-1	-1
S2	-1	0
S3	-1	1
S4	0	-1
S5	0	0
S6	0	1
S7	1	-1
S8	1	0
S9	1	1

Where, X_1 code for tween 80 w/v and X_2 code for drug: GMS ratio.

2.5 CHARACTERIZATION

2.5.1 *FT-IR studies*

FT-IR studies were carried out for pure drug alone and along with excipients. The pure drug, excipients and PLGA Nano particles of drug were crushed differently with KBr and their IR spectra were recorded over the region 400 to 4000 cm^{-1} in FTIR 8400S, Shimadzu instruments.[6]

2.5.2 Differential scanning calorimetry (DSC) analysis

Differential Scanning Calorimetric (DSC) is among the most useful tools of thermal analysis available for the determination of various thermal parameters of a formulation, which allows for the detection of phase transition of the samples under study.

DSC scans of the powdered sample of DST and PLGA nano particles of DST were recorded using DSC-Shimadzu 60 with TDA trend line software. The thermal traces were obtained by heating from 50°C to 300°Cat heating rate of 10°C under inert N_2 dynamic atmosphere ($100ml.min^{-1}$) in open crucibles. Aluminium pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpic response.

2.5.3 Particle size

The average diameter, polydispersity index (PI) and zeta potential of dry nanoparticles were determined by Malvern nano HSA 3000 (Malvern Instruments Ltd., U.K) at room temperature. The samples were adequately diluted with deionized water and placed in a cell. The average particle size was measured after performing the experiment in triplicates.[7]

2.5.4 *In-vitro* drug release study

In vitro release studies were performed using dialysis bag method having molecular weight of 12,000–14,000 Daltons. PLGA Nanoparticles dispersion equivalent to 20 mg of drug was filled into a dialysis membrane bag and tied at both the ends and placed in a basket containing 1000 ml of diffusion medium; temperature and speed were maintained at 37 ± 2^0C and 60rpm, respectively, using dissolution tester (TDT-06P Tablet Dissolution Rate Test AppElectro. Lab, Mumbai) by USP apparatus I (basket) in 1000ml 1%(w/v) triton X 100 pH 4 acetate buffer. At fixed intervals of 0.5, 1, 2,3 ,5,7,9,11,15,19,23,28,32,36,40,44 hrs.10 ml of sample was withdrawn from release media and analyzed by using UV method as described above. Cumulative percentage release was then calculated from the amount of drug release.[7]

2.5.5 Powder X-ray diffraction (PXRD)

PXRD diffractograms of the pure drug and spray dried PLGA nanoparticles were recorded. The theta scan range was 0 to 50 theta.

2.5.6 Solubility Study

Accurately weigh pure DST drug and equivalent amount of PLGA nanoparticles. Transfer it in 100 ml of volumetric flask and dissolve it with distilled water. Dilute the solutions as per need. Take absorbance by using UV-spectrophotometer. Determine the solubility of pure DST drug & PLGA nanoformulations. Find out % increment in Solubility also.[7]

2.5.7 Zeta Potential

The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed.

2.5.8 Measurement of drug Entrapment Efficiency and Loading Capacity

Samples from each PLGA nanoparticles were centrifuged at 10,000 rpm and 4°C for 20 minutes using an Optima MAX-E ultracentrifuge (Beckman Coulter, Inc., Nyon, Switzerland). The amount of un entrapped Drug in the supernatant obtained after centrifugation was determined using UV. The percent entrapment efficiency

(%EE) and percent drug loading (%DL) were calculated according to Equations.[7]

$$\%\ EE = \frac{W_{TotalDrug} - W_{FreeDrug}}{W_{TotalDrug}} X\ 100$$

$$\%\ LC = \frac{W_{TotalDrug} - W_{FreeDrug}}{(W_{TotalDrug+W_{TotalLipid}} - W_{FreeDrug})} X\ 100$$

2.5.9 SEM Study

SEM Study is mainly done to get idea about surface morphology of the solid Particles. SEM study is very useful to observe nano/micro size particulate structure.

2.6 RESULTS AND DISCUSSION

2.6.1 Results and Discussion of preliminary batches for PLGA Nanoparticles

2.6.1.1 Selection of Surfactant

Six batches were prepared with different surfactant and their concentrations are as shown in below Table.

Table 5: Formulation Optimization for PLGA Nanoparticles.

Batch No.	Drug (mg)	Stabilizers	PLGA polymer (mg)	Millipor water (ml)	Organic solvent (ml)
PS1	20	Tween 80 (1% W/V)	20	25	10
PS2	**20**	**Tween 80 (1.5% W/W)**	**20**	**25**	**10**
PS3	20	Tween 80 (2% W/W)	20	25	10
PS4	20	Poloxamer-188(1 % W/W)	20	25	10
PS5	20	Poloxamer-188 (1.5 % W/W)	20	25	10
PS6	20	Poloxamer-188 (2 % W/W)	20	25	10

1.5% w/v of Tween 80 showed better results in terms of precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.6.1.2 Selection of Homogenization Speed

Three batches were prepared with different homogenization speed at 10000, 15000, 20000 rpm as shown in below table.

Table 6: Optimization of homogenization speed for PLGA Nanoparticles

Batch No.	Homogenization speed(RPM)
PS2 (A)	10000
PS2 (B)	**15000**
PS2 (C)	20000

15000 rpm showed good results compare to other and utilize for further batches.

2.6.1.3 Results of Preliminary batches for selection of Homogenization time.

Three batches were prepared with different homogenization time at 20, 30, 40 min as shown in below table.

Table 7: Optimization of homogenization time for PLGA Nanoparticles.

Batch No.	Homogenization Time in (mins)
PS2 (D)	20
PS2 (E)	**30**
PS2 (F)	40

30 min as homogenization time showed good results compare to other and utilize for further batches.

2.6.1.4 Selection of Pressure using High Pressure Homogenizer (HPH) (Avestine)

Three batches with 5000, 10000, 15000 psi were prepared as shown in below table.

Table 8: Pressure Optimization Table for PLGA Nanoparticles (HPH)

Batch No.	Drug (mg)	Polymer (mg)	Pressure (Psi)	Cycles
PS2(G)	20	20	5000	10
PS2(H)	**20**	**20**	**10000**	**10**
PS2(I)	20	20	15000	10

10000 psi showed satisfactory results compare to other and utilize for further batches.

2.6.2 Results and Discussion of preliminary batches for SLN

2.6.2.1 Selection of Surfactant

Six batches were prepared with different surfactant and their concentration as shown in below table.

Table 9: Formulation Optimization Table of SLNs.

Batch No.	Drug (mg)	Stabilizers	GMS (mg)	Millipor water (ml)	Organic Solvent (ml)
PG1	20	Tween 80 (1% W/V)	20	25	12
PG2	20	Tween 80 (1.5% W/W)	20	25	12
PG3	20	Tween 80 (2% W/W)	20	25	12
PG4	20	Poloxamer-188(1 % W/W)	20	25	12
PG5	20	Poloxamer-188 (1.5 % W/W)	20	25	12
PG6	20	Poloxamer-188 (2 % W/W)	20	25	12

1.5% w/v of Tween 80 shown better results in terms of precipitation, particle size in Leica microscope, bluish color and redispersibility.

2.6.2.2 Selection of Homogenization speed

Three batches were prepared with different homogenization speed at 10000, 15000, 20000 rpm as shown in below table.

Table 10: Optimization of Homogenization speed for SLNs.

Batch No.	Homogenization speed(RPM)
PG2 (A)	10000
PG2 (B)	**15000**
PG2 (C)	20000

15000 rpm shown good results compare to other and utilize for further batches.

2.6.2.3 Selection of Homogenization time

Three batches were prepared with different homogenization time at 15, 30, 45 min as shown in below Table.

Table 11: Optimization of homogenization time for SLNs.

Batch No.	Homogenization Time in (mins)
PS2 (D)	15
PS2 (E)	**30**
PS2 (F)	45

1.5% w/v of Tween 80 shown better results in terms of precipitation, particle size in leica microscope, bluish color and redispersibility.

2.6.3.2 Selection of Homogenization speed

Three batches were prepared with different homogenization speed at 10000, 15000, 20000 rpm as shown in below table.

Table 14: Optimization of homogenization speed for Nanocrystals

Batch No.	Homogenization speed(RPM)
NC2 (A)	10000
NC2 (B)	**15000**
NC2 (C)	20000

30 min as homogenization time shown good results compare to other and utilize for further batches.

2.6.2.4 Selection of Pressure using High Pressure Homogenizer (HPH) (Avestine)

Three batches with 5000, 7500, 10000 psi were prepared as shown in below table.

Table 12: Pressure Optimization Table for SLNs (HPH).

Batch No.	Drug (mg)	GMS (mg)	Pressure (Psi)	Cycles
PG2(G)	20	20	5000	10
PG2(H)	**20**	**20**	**7500**	**10**
PG2(I)	20	20	10000	10

7500 psi shown satisfactory results compare to other and utilize for further batches.

2.6.3 Results and Discussion of preliminary batches for Nanocrystals

2.6.3.1 Selection of surfactant

Three batches were prepared with different surfactant and their concentration as shown in below table.

Table 13: Formulation Optimization Table of Nanocrystals.

Batch No.	Drug (mg)	Stabilizers	Millipore water (ml)	Organic solvent (ml)
NC1	20	Tween 80 (1% W/V)	25	7
NC2	**20**	**Tween 80 (1.5% W/W)**	**25**	**7**
NC3	20	Tween 80 (2% W/W)	25	7

15000 rpm shown good results compare to other and utilize for further batches.

2.6.3.3 Selection of Homogenization time

Three batches were prepared with different homogenization time at 15, 30, 45 min as shown in below table.

Table 15: Optimization of homogenization time for Nanocrystals

Batch No.	Homogenization Time in (mins)
NC2 (D)	15
NC2 (E)	**30**
NC2 (F)	45

30 min as homogenization time shown good results compare to other and utilize for further batches.

2.6.3.4 Selection of Pressure using High Pressure Homogenizer (HPH) (Avestine)

Three batches with 8000, 10000, 12000 psi were prepared as shown in below table.

Table 16: Pressure Optimization Table for Nano crystals (HPH)

Batch No.	Drug (mg)	Pressure (Psi)	Cycles
NC2G)	20	8000	10
NC2(H)	**20**	**10000**	**10**
NC2(I)	20	12000	10

10000 psi shown satisfactory results compare to other and utilize for further batches.

2.6.4 FT-IR Study

FT-IR spectroscopy was carried out to study drug-polymer interaction. There are some characteristic peaks of the drug (DST) between IR spectrum of DST was identified by absorption peaks at 3300 cm^{-1} (secondary amine N-Hstretch), 3070 cm^{-1} (=C-H aromatic ring), 2958 cm^{-1} (C-H stretch), 1150 cm^{-1} (C-N stretch), 1689 cm^{-1} (C=O stretch) and 1595 cm^{-1} (C=C) stretch, aromatic ring. For PLGA, one broad peak is observed at ~2100 cm^{-1}. Physically mixed sample of drug and PLGA proved that PLGA & tween 80 is not affecting the characteristic peaks of the Drug. Also In SLNs due to GSM drug peak did not changed. Also tween 80 does not cause change in nano crystal peak. The same thing is further proven by DSC thermogram of same physical mixture.

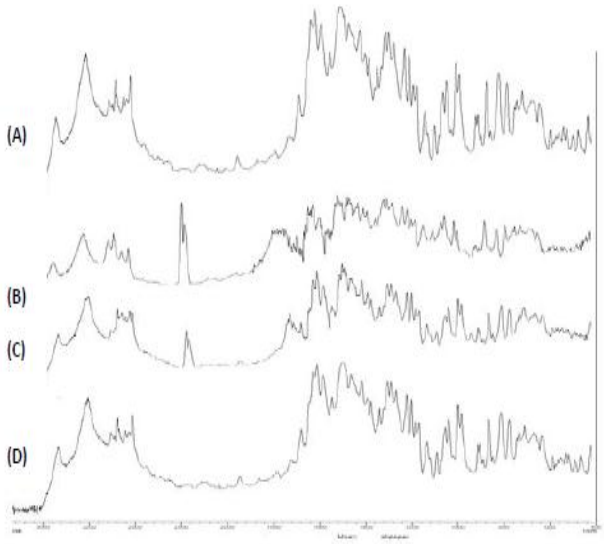

Figure 3: FT-IR Spectra of (A) DST Drug (B) PLGA Nanoparticles (C) SLNs (D) Nanocrystals.

2.6.5 DSC Study

DSC thermogram study was carried out for checking interaction between excipients. Pure drug showed endothermic peak at 288.20 ˚C while PLGA nanoparticles showed peak at 290.01 ˚C.SLNs showed peak at 290.87 ˚C. In case of SLNs, there was no interaction between GMS & DST. Nanocrystals showed peak at 285.20 ˚C. In Nanocrystals there was no interaction between drug and tween 80.

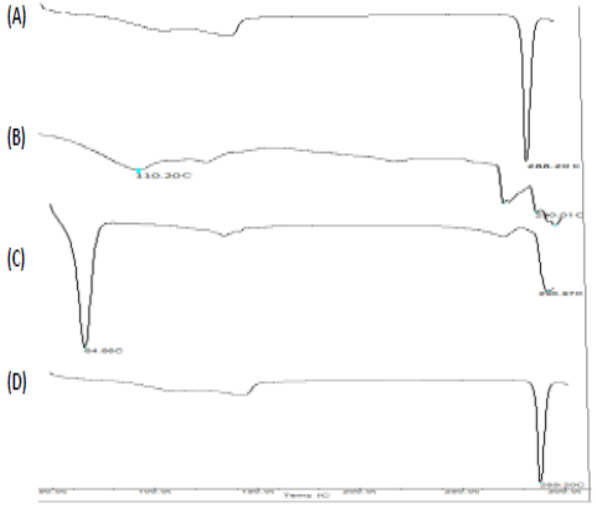

Figure 4: DSC Spectra of (A) DST Drug (B) PLGA Nanoparticles (C) SLNs (D) Nanocrystals.

2.6.6 Particle Size Analysis

Droplet size analysis was done by Malvern nano HSA 3000 (Malvern Instruments Ltd., U.K). It measures the size of particles typically in the sub-micron region. It measures Brownian motion and relates this to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surrounds them. The measurement by Malvern is done by Laser Scattering Principle as per Mie Theory and Fraunhofer Approximation. Malvern measure the intensity of light scattered by nanoparticles.

It was seen that PLGA nanoparticles had best particle size (P7 batch) in Nano range. In SLNs there was comparatively larger size than PLGA nanoparticles. In SLNs better particle size was seen in S7 Batch. While in nanocrystals doesn't showed good results with particle size. In all batches of nanocrystals, C3 batch showed comparatively better results (652.04). C3 had high Particle Size (652.04 nm) than P7. PDI was best in case of P7 Compared to S7 & C3. So it showed that PLGA nanoparticles were in monodisperse form.

Table 17: Particle size of diluted sample of DST Nano formulations in water

Batch No.	Stabilizers	Particle Size (nm)	PDI
P7	Tween 80(2 % W/W)	275.7±0.3	0.203
S7	Tween 80(2 % W/W)	686.7±0.2	0.221
C3	Tween 80(2 % W/W)	579.5±0.4	0.408

2.6.7 Drug Entrapment Efficiency and drug Loading.

Drug loading & Entrapment efficiency were measured in PLGA nanoparticles & SLNs. In Case of PLGA nanoparticles P4 Batch have high % drug loading while P7 batch showed High in entrapment efficiency. SLNs showed drug loading & Entrapment as 44.89% & 86.72% respectively. In SLNs batch S4 had high % drug loading capacity while S7 showed high Entrapment efficiency and it was 41.98% && 76.78% respectively. So, PLGA nanoparticles had high in % drug loading as well as entrapment efficiency.

Table 18: % Drug Loading & Entrapment efficiency of Nanoformulations.

Batch No.	% Drug Loading	Entrapment Efficiency
P7	43.88±0.3	86.72±0.5
S7	20.72±0.7	76.78±0.4

2.6.8 *In-Vitro* Drug Release Study

In vitro drug release study we earned that PLGA nanoparticles (P7) have sustained effect than SLNs & Nanocrystals. PLGA NP showed 79.45% release at 44 hrs. SLNs have cumulative % drug release were 98.75 % for S7 batch at 44 hr, while for nanocrystals, it was 98.86% release with C3 at 44 hrs. So PLGA nanoparticles prepared by High Pressure homogenizer had best sustain drug release.

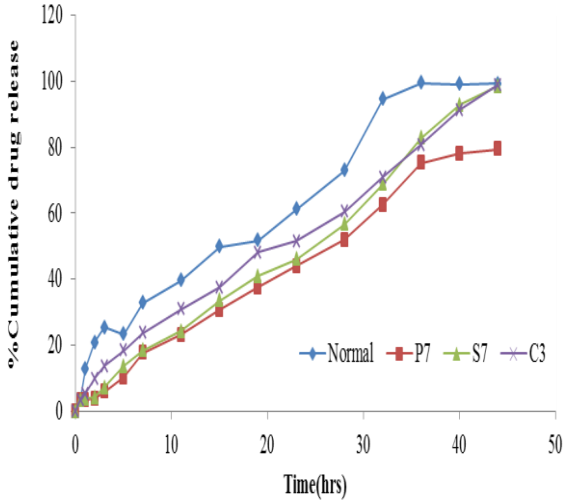

Figure 5: *In-Vitro* Drug release of nanoformulations.

2.6.9 Solubility Study

Pure DST drug had 0.078 mg/ ml of solubility, In solubility study came to know that P7 (PLGA nanoparticles) had high increment in solubility than others. SLNs had good solubility increments than nanocrystals. SLNs had poor Solubility increments than PLGA nanoparticles. This Could also revealed from XRD pattern of PLGA Nanoparticles. So PLGA nanoparticles Prepared by High pressure homogenizer had high solubility than other Nano formulations.

Table 19: Solubility of Nanoformulations

Batch No.	Solubility (mg/ml)	% Solubility Increment
Pure Drug	0.078mg/ml±0.007	-
P7	0.160mg/ml±0.06	205.12%
S7	0.145mg/ml±0.04	185.89%
C3	0.138mg/ml±0.04	176.92%

2.6.10 Zeta Potential

The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. All nanoformulations P7, S7 & C3 had good zeta potential values. So it indicated that all prepared nanoformulations had comparatively effective stability.

Table 20: Zeta Potential of Optimized Nanoformulations

Batch No:	Zeta Potential
P7	8.53
S7	-21.1
C3	-22.1

2.6.11 X-Ray Diffraction (XRD) Study

XRD was done to check crystalline transition of nanoformulations. From above overlay Spectra revealed that pure DST had sharp intense peak. So it showed its crystalline nature. Nanocrystals had decreased intensity than pure drug. It shows that conversion of crystalline to amorphous form. SLNs (C3) had also decrease in peak than pure drug. PLGA nanoparticles had very broad peak and it showed highly conversion into amorphous form. PLGA nanoparticle had amorphous form. It could also reveal from Solubility data. PLGA Nano particle had high solubility & Broad peak than other nanoformulations.

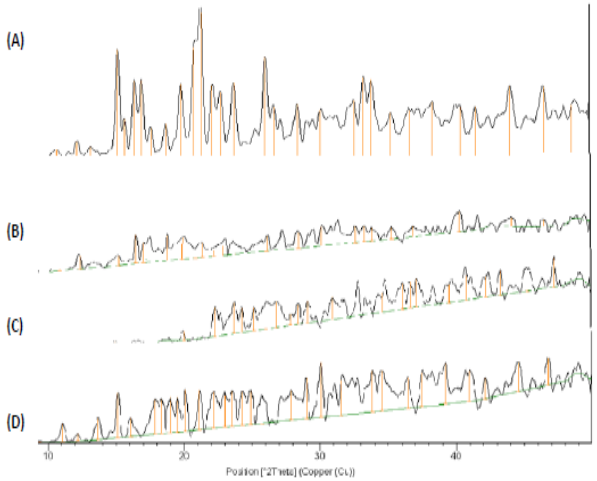

Figure 6: XRD Spectra of (A) DST (B) PLGA Nanoparticles (C) SLNs (D) Nanocrystals

2.6.12 Scanning Electron Microscope (SEM) Study

The morphological characters of DST nanoformulations are shown in below figure. It was observed that the nature of PLGA nano particles appears to be homogeneous, smooth and spherical in shape which is confirmed by surface morphology. The particles have moderate uniformity and all the particles were discrete entities, shows aggregation of particles after lyophilization and these particles were readily redispersible. It was clear from image that DST nib loaded SLNs were spherical in shape with the presence of some particle aggregates. The sizes observed from SEM micrographs were slightly higher than those obtained from particle size analyser. The nanocrystals have shown spherical shape. The SEM of the DST nanocrystals showed that the nanocrystals have a solid dense structure with smooth spherical shape. The pure drug show irregular shape and rough surface.

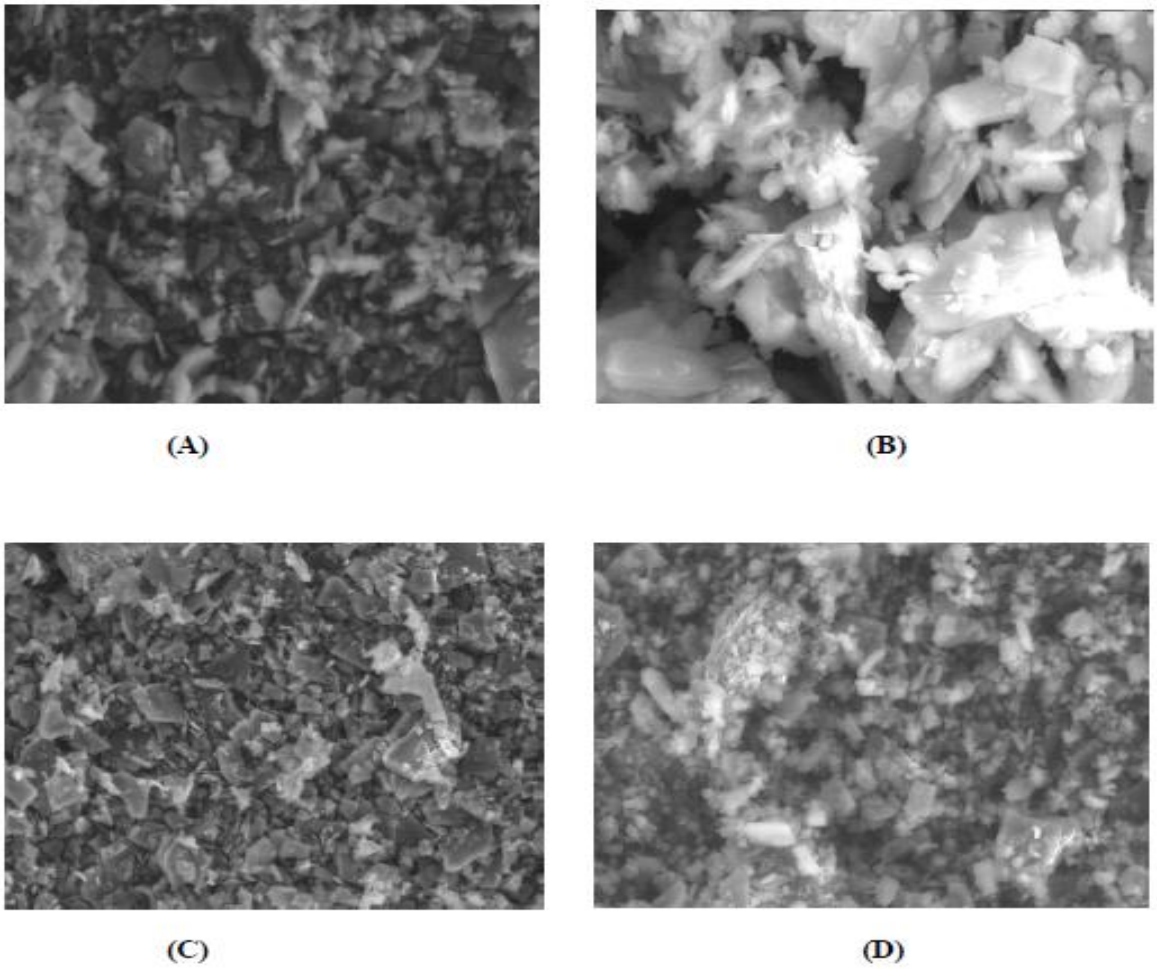

Figure 7: SEM images of (A) PLGA nanoparticles (B) DST Pure Drug (C) SLNs (D) Nanocrystals

3 CONCLUSION

In the present study, a poorly aqueous-soluble drug DST was successfully incorporated into PLGA NP, SLNs and nanocrystals by High speed homogenization and high pressure homogenization method using tween 80 as a stabilizer and GMS as a lipid. The prepared nano formulations exhibited nanometer range spherical and amorphous with *in vitro* sustained release profile. Comparison of saturated solubility parameter of the nano formulations with plain drug showed that Nano formulations improve the solubility of a drug to a significant extent. This increase in the solubility can lead to increase in bioavailability and reduction in the dose required for therapeutics action. This can lead to fewer side effects and overall decrease in the cost of therapy. Among all nano formulations PLGA NP provided better results in terms of particle size, %entrapment efficiency, in vitro drug release and XRD compare to other nano formulations (SLNs, nano crystals). Thus it can be concluded that the nano formulations are suitable carriers for improving the solubility which can lead to improve oral bioavailability of DST.

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