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Original Article

FORMULATION AND EVALUATION OF POORLY SOLUBLE ETRAVIRINE BY SPRAY DRYING METHOD

K. RAMESH[1, 3*], BONAGIRI CHANDRASHEKAR[2] ,PODILE KHADGAPATHI[3]

[1]JNTUH, Hyderabad 500072, Telangana, India, [2]Bomma Institute of Pharmacy, Allipuram, Khammam-507318, Telangana, India, [3]Hetero Labs Ltd, Hyderabad 500055, Telangana, India
Email: rameshsuper6@gmail.com

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ABSTRACT

Objective: Current research work deals with the enhancement of solubility and dissolution of Poorly soluble Etravirine, which is belongs to BCS class-IV by Spray drying method (SD), which has been found to be the most widely used method in enhancing the solubility and rate of dissolution of poorly soluble drug substances.

Methods: Etravirine is belongs to BCS class-IV. It is insoluble in water and practically insoluble across the pH range of 1 - 6.8 of the major concerns with the Etravirine is its poor solubility, which results into poor bioavailability after oral administration. SD method has been proven to be an efficient method for conversion of poorly soluble crystalline polymorphic form into highly soluble amorphous form and make it useful for formulating lab scale batches to commercial batches. Hence, Spray drying method was chosen for converting poorly soluble crystalline form of Etravirine into highly soluble amorphous form using various carriers like Soluplus and Povidone (drug to polymer in 1:2 and 2:1 ratios) to increase its aqueous solubility and rate of drug release.

Results: There is almost 4 fold and 15 fold increases in the solubility of Etravirine prepared by SD as compared with Etravirine (crystalline form) in purified water and in pH 6.8 Phosphate buffer, respectively. From the characterization of spray dried powder of SD4 by Fourier Transform Infrared Spectroscopy **(**FT-IR), Differential Scanning Calorimetry **(**DSC), Powder X-ray diffraction (p-XRD), Scanning electron microscopy **(**SEM) concluded that crystalline Etravirine has been converted into an amorphous form. Faster and high drug release was found in the formulation of SD4.

Conclusion: The obtained results suggested that Spray drying method might be an efficacious approach for converting poorly soluble crystalline polymorphic form into highly soluble amorphous form and ultimately results in enhancing the therapeutic potential of Etravirine.

Keywords: Etravirine, Spray drying method, Soluplus, Povidone, Amorphous form.

INTRODUCTION

The oral route of administration is the most preferred and widely acceptable route of delivery due to ease of ingestion for many drugs. Solubility is one of the important parameter to achieve the desired concentration of drug in systemic circulation for the pharmacological response to be shown. Drug efficacy can be severely limited by poor aqueous solubility [1]. Etravirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI), in combination with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in antiretroviral treatment-experienced patients ages 6 years and older, who have evidence of viral replication and HIV-1 strains resistant to a non-nucleoside reverse transcriptase inhibitor (NNRTI) and other antiretroviral agents [2]. It is off-white, non-hygroscopic, crystalline powder. Etravirine is very slightly soluble in water (0.07 mg / ml, at 25 **°C**) and practically insoluble in pH 6.8 Phosphate buffer (0.01 mg / ml, at 25 **°C).** Based on the literature, Etravirine is found to be low to moderate permeability [3]. The bioavailability of Etravirine is not documented anywhere.

A poorly water soluble drug with poor solubility and low dissolution rate in *in-vivo* is a reason for its poor bioavailability. Solubility of such drugs can be improved by incorporating the drug in a matrix of hydrophilic carrier(s) obtaining a product called a solid dispersion. Limited aqueous solubility of the active pharmaceutical ingredient can result in poor bioavailability, which is a major issue for the pharmaceutical industry [1, 4].

Poor solubility and bioavailability, of an existing or newly synthesized drug always pose challenge in the development of efficient pharmaceutical formulation. Numerous technologies can be used to improve the solubility and among them amorphous solid dispersion based spray drying technology can be successfully useful for development of the product from lab scale to commercial scale with a wide range of powder characteristics [5].

Spray drying is an efficient technology for solid dispersion manufacturing since it allows extreme rapid solvent evaporation leading to fast transformation of a drug-carrier solution to solid drug-carrier particles. Solvent evaporation kinetics certainly contribute to the formation of amorphous solid dispersions, but also other factors like the interplay between the drug, carrier and solvent, the solution state of the drug, formulation parameters (e. g. feed concentration or solvent, type) and process parameters (e. g. drying gas flow rate or solution spray rate) will influence the final physical structure of the obtained solid dispersion particles [6].

Nano spray drying is a novel one-step granulation technology for the preparation of micro to nano particles with narrow size distribution. The biologically active molecule can maintain its structure and activity because of the mild preparation process. So this technology is suitable for the preparation of thermo sensitive and sparingly soluble drugs [7]. The use of amorphous solid dispersions is an interesting strategy to increase the bioavailability of poorly soluble drugs by improving their rate and extent of dissolution. Lack of understanding of the physical chemistry and their *in vivo* behavior still hamper full breakthrough in pharmaceutical industry [8].

Spray drying is a particle processing technology that transforms a liquid feed stock into a powder product by first spraying the feed stock to create droplets, and then evaporating the feed stock liquid through the use of a heated drying medium, typically air. The liquid feed stock can take the form of a solution, suspension, or emulsion, and must be easily pumpable and capable of droplet formation [9].

The objective of the present study was to convert crystalline form of Etravirine into an amorphous form, improve the solubility and drug release rate by Spray drying of crystalline Etravirine with hydrophilic polymer/polymer mixtures, like Soluplus a novel polymer with amphiphilic properties, and Povidone (Kollidon 30) [10].

MATERIALS AND METHODS

Materials

INTELENCE® (Etravirine) 200 mg tablets were obtained from Tibotec Pharmaceuticals Ltd, manufactured by Janssen Cilag S. p. A., Latina, Italy. Etravirine (crystalline) drug substance was gifted by Hetero Drugs Ltd, Hyderabad, India. Soluplus and Povidone (Kollidon 30) were gifted by BASF, USA; microcrystalline cellulose (Avicel PH105) was gifted by FMC Biopolymer, U. S. Hard gelatine capsules were gifted by ACG Associated Capsules, Mumbai, India. All other solvents used were of analytical grade.

Methods

Formulation of Etravirine by Spray drying method

1. Etravirine was dissolved in solvent mixtures of Dichloromethane and Acetone (6:4), a clear solution was formed.

2. In first set of formulation Povidone (Kollidon 30) / in the other set of formulation Polyvinyl-Caprolactam-Polyvinyl Acetate-Polyethylene Glycol Graft Copolymer (Soluplus) was added to the above drug solution and stirred well till to get a clear solution.

3: Microcrystalline cellulose, grade Avicel PH105 was dispersed in the above solution. The above dispersion was subjected to spray drying using BUCHI spray dryer (Inlet air temperature 60 – 70 °C, Aspiration 90 – 100 %; Nozzle tip: 0.2 mm; Nitrogen gas cylinder). The composition is shown in table 2.

The particle size of microcrystalline cellulose was selected such that when mixed into the solution of active drug substance and hydrophilic polymer should not block / clog the atomizer, should be easily atomizable. The density of the resulting spray dried powder will be increased with the addition of Microcrystalline cellulose, it also helps in improvement of flow properties of resulting spray dried powder, may also function to increase the propertied like compressibility, disintegration and dissolution of the resulting spray-dried powder of Etravirine.

The majority of the spray dried powder was collected in the drying chamber cylinder with aspiration below 90 % and it was found to be coarser powder as compared to spray dried powder, which was collected in Extraction cyclone cylinder where aspiration above 90% to 100%. Spray dried powder was found to be coarser with nozzle size more than 0.4 mm, coarser grade powder was collected in drying chamber cylinder. The parameters are depicted in table 1 and composition details are presented in table-2.

Table 1: Parameters considered during spray drying

Atomizer qualifications	
Nozzle tip	0.2 mm
Nozzle diameter	0.4 mm
Cap diameter	1.4 mm
Spray drying parameters	
Inlet temperature	60 – 70 **°C**
Pump rate for spraying dispersion	25 – 35 %
Nitrogen gas pressure	30 – 40 mm Hg

In the first set of formulation, spray drying of dispersion comprising Etravirine. Povidone and Microcrystalline cellulose in 1:2:0.5 and 2:1:1 and in the second set of formulation, spray drying of dispersion comprising Etravirine, Soluplus and Microcrystalline cellulose in 2:1:1 and 1:2:0.5 was performed. In the present investigation 4 formulations were prepared and their complete composition is shown in table 2**.** The resulting spray dried powder (fig. 1 and 2) was filled in hard gelatine capsules and evaluated for dissolution profiles.

Table 2: Composition of Etravirine by Spray drying method (SD)

S. No.	**Ingredients↓**	**SD1 (mg / unit)**	**SD2 (mg / unit)**	**SD3 (mg / unit)**	**SD4 (mg / unit)**
1.	Etravirine	200.0	200.0	200.0	200.0
2.	Povidone (Kollidon 30)	400.0	100.0	x	x
3.	Soluplus	x	x	100.0	400.0
4.	Microcrystalline cellulose (Avicel PH 105)	100.0	100.0	100.0	100.0
5.	Dichloromethane (6 parts)	q. s	q. s	q. s	q. s
6.	Acetone (4 parts)	q. s	q. s	q. s	q. s

The resulting spray dried mixture was determined for drug substance solubility. The resulting spray dried powder shown good solubility and were further studied for the % assay of drug and *in-vitro* drug release studies.

Fig. 1: Spray dried powder of SD2

Fig. 2: Spray dried powder of SD4

Solubility studies of spray dried powder of Etravirine

The spray dried powder of Etravirine with Povidone (Kollidon 30) (SD1 and SD2) and with Soluplus (SD3 and SD4) were added to 25 ml of volumetric flask, filled with purified water / pH 6.8 Phosphate buffer and sealed them. Samples were shaken for the 48 hours at room temperature. Subsequently, the suspensions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Etravirine by UV/Visible spectrophotometer at λ_{max} of 235 nm.

Characterization

Fourier Transform Infrared Spectroscopy (FTIR) [11]

FTIR spectra of Etravirine (crystalline form), physical mixture and spray dried powder of SD4 formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR affinity-1 Spectrophotometer. The IR spectrum of the samples was prepared using KBr (spectroscopic grade) disks by means of hydraulic pellet press at pressure of 7 to 10 tons.

Differential Scanning Calorimetry (DSC) [12, 13]

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5 °C/min, over a temperature range of 0 **°C** to 250 **°C.**

Powder X-ray diffraction [12, 13]

A Bruker D8 diffractometer was used to perform powder X-ray diffraction (PXRD) of all samples. A Cu K-α 1 tube was the source, set at 40 KV and 50mA. A scan from 2 to $60^0$2 θ was carried out at a rate of 0.01220^0 2θ/s. The diffractometer was calibrated using powdered α-alumina. Spray dried powder of SD4 samples was ground before analysis.

Scanning electron microscopy [12, 13]

The shape and surface morphology of the Etravirine and hot melt extrudes of SD4 were examined using XL 30 model JEOL 6800 scanning electron microscope (Japan).

Assay of Spray dried powder

Weight equivalent to ten units was dissolved in 100 ml of Methanol. The samples were sonicated for 30 minutes. The hazy dispersion was filtered through a Whatman filter paper no 1. Subsequently, the filtered solution was diluted further with Methanol and were analyzed for the Etravirine by UV/Visible spectrophotometer at λ_{max} of 235 nm.

***In vitro* drug release profiles** [14]

The *in vitro* drug release profiles for Etarvirine (crystalline form), Spray dried powder (SD1 to SD4) filled in hard gelatine capsules size "3" and corresponding Innovator product (Intelence® 200 mg tablets) was performed using USP type 2 dissolution apparatus and the dissolution conditions are shown in the table 3. The samples were withdrawn at specified time intervals and the obtained samples were analyzed by using UV/Visible spectrophotometer at 235 nm. The cumulative percentage release was calculated.

RESULTS

Fourier transform-infrared (FT – IR) studies

FT-IR spectrums are intended to determine if there is any interaction between the drug and the excipient used. The formulation SD4 (fig. 4) shown the characteristic peaks at wave numbers close to that of crystalline form of Etravirine (fig. 3). There was no alteration in the characteristic peaks of in the spray dried powder of SD4, indicates that there was no interaction between the drug and polymer.

Table 3: *In Vitro* dissolution studies test parameters

Instrument	Electro lab-USP type 1 dissolution test apparatus.
Dissolution medium	1.0 % Sodium lauryl sulfate (SLS) in 0.01 M HCl in two phases: Phase 1: 500 mL of degassed 0.01 M HCl (For 10 minutes). Phase 2: Add 400 mL of 2.25 % SLS in 0.01 M HCl after 10 minutes.
Apparatus	USP apparatus – I (Basket type)
Temperature	37±0.5 °C
RPM	100
Volume of medium	900 ml
Sampling intervals	5, 10, 15, 30, 45, 60 and 90 minutes
Sample volume	10 ml withdrawn and replaced with 10 ml of dissolution medium

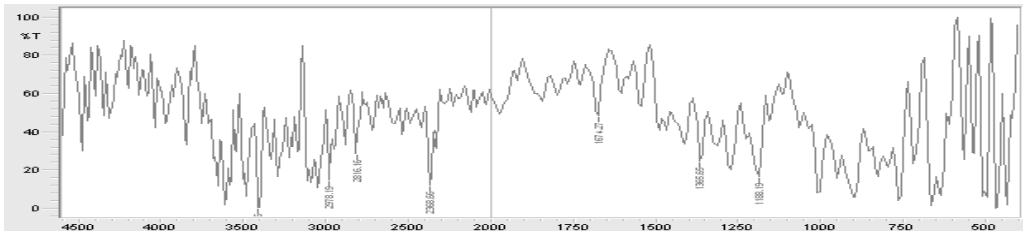


Fig. 3: FTIR spectra of crystalline form of Etravirine

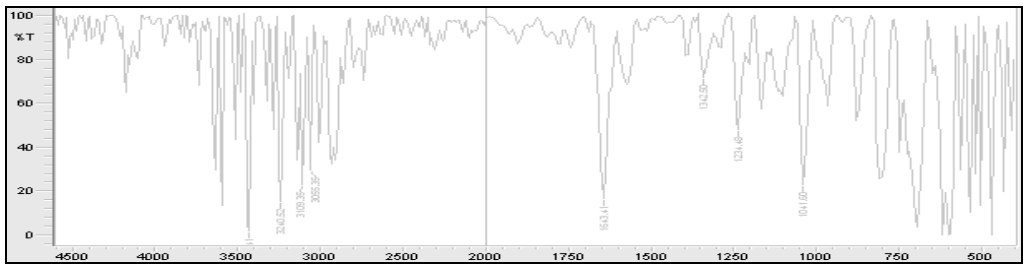


Fig. 4: FTIR spectra of formulation SD4 prepared by spray drying method

Differential scanning calorimetry (DSC)

The DSC thermograms of Etravirine presented in fig. 5, sharp endothermic peak at melting point (265 °C), indicating that the Etravirine is crystalline in nature. The absence of drug peak in the spray dried powder of SD4 (Etravirine: Soluplus: Microcrystalline cellulose (1:2:0.5)) indicating that the crystalline form of Etravirine has been converted into an amorphous form.

XRD Analysis

The diffractogram of Etravirine consist of sharp multiple peaks, indicating the crystalline nature of the drug. In case of spray dried powder of SD4 when exposed to X-ray beam, disappearance of all characteristic peaks of Etravirine (fig. 6). This indicates complete transformation of crystalline Etravirine into amorphous form by Spray drying process.

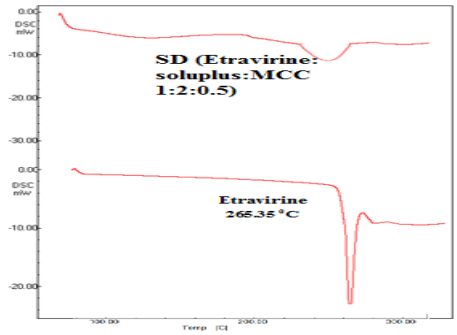
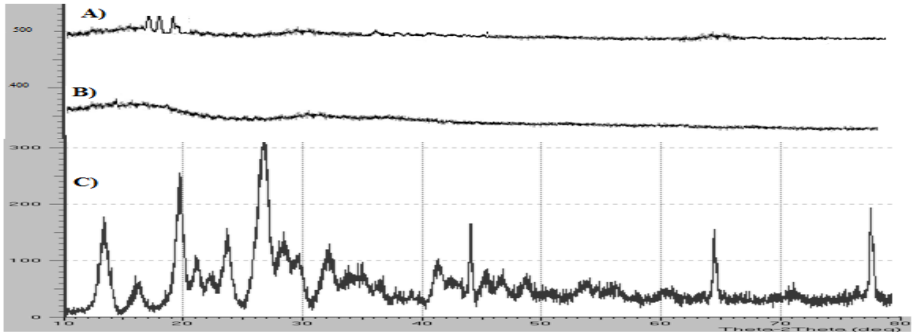
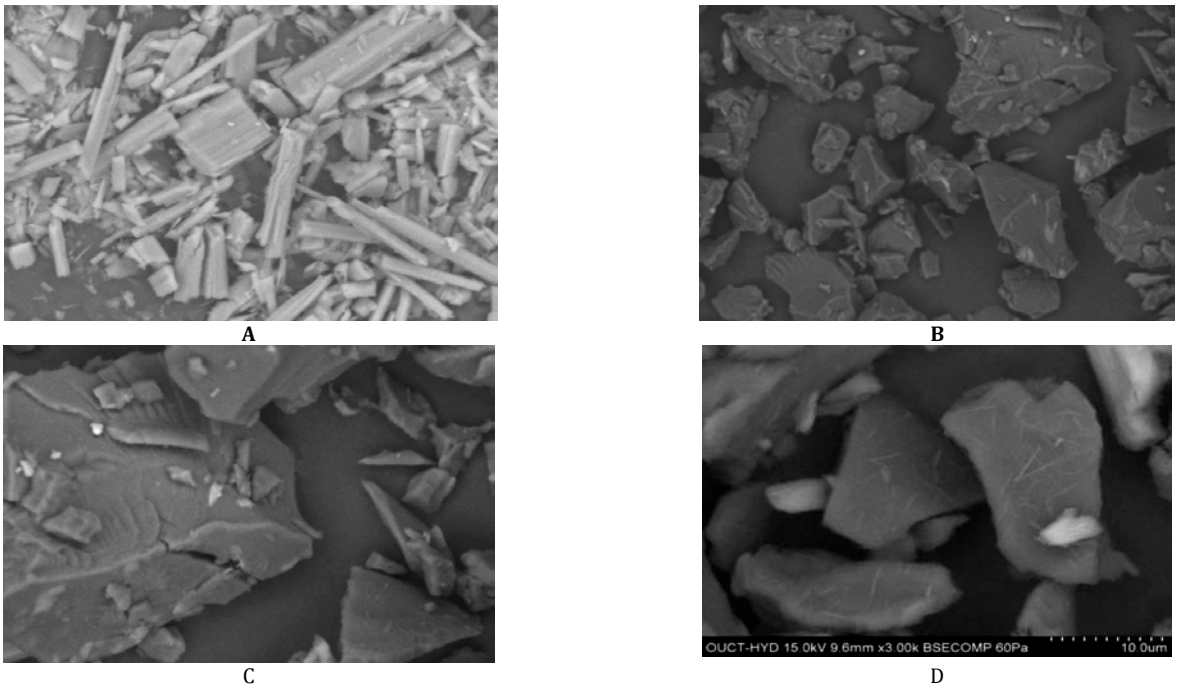


Fig. 5: DSC thermograms of crystalline form of Etravirine and spray dried powder of SD4



A) Spray dried powder of SD4 B) Placebo C) Crystalline form of Etravirine

fig. **6: Powder X-ray diffraction patterns of Crystalline form of Etravirine, placebo and formulation SD4**



A B C D

Fig. 7: SEM images of Etravirine Crystalline form of Etravirine (A), spray dried powder of SD4 (B, C & D)

Scanning electron microscopy (SEM)

Surface micrographs of Etravirine and spray dried powder of SD4 were analysed using SEM technique. The SEM micrograph of Etravirine showed large crystalline forms of drug agglomerates with ordered shape and size (fig. 7A). The surface characteristics of spray dried powder of SD4 (fig. 7B, C & D) showed rough disordered and intact structures, indicated that crystalline form of Etravirine has been converted into an amorphous form.

Solubility studies of spray dried powder of Etravirine

Four formulations were prepared by spray drying method with their respective carrier with Micro crystalline cellulose. The resulting spray dried powder was analyzed for solubility of the drug

substance and was compared with the crystalline form of Etravirine itself. The formulation SD4 with Soluplus in the ratio of 1:2 (drug to carrier) which had shown increased solubility by almost 4.0 fold in Purified water and more than 15 folds in pH 6.8 Phosphate buffer as compared to that of the solubility of crystalline form of Etravirine. The results are tabulated in table 4.

% Assay of drug

% assay of drug in all 4 formulations are presented in table 5. The % assay of the drug was found to be in the range of 89.2 % - 95.1 %. Maximum % drug content i.e. 95.1 % was found in the formulation SD4.

Table 4: Solubility of spray dried powder

S. No.	Formulation	Solubility in water (mg/ml)	Solubility in 6.8 Phosphate (mg/ml)
1.	Etravirine	0.07±0.03	0.01±0.01
2.	SD1	0.22±0.08	0.10±0.04
3.	SD2	0.24±0.05	0.12±0.03
4.	SD3	0.26±0.11	0.13±0.08
5.	SD4	0.28±0.06	0.15±0.04

Table 5: % Assay for different formulations

S. No.	Formulation	% Drug content
1.	SD1	89.2±2.4 %
2.	SD2	91.6±3.3 %
3.	SD3	93.3±2.7 %
4.	SD4	95.1±2.2 %

***In vitro* dissolution studies**

The cumulative percentage drug release of formulation SD1 to SD4 as a function of time are tabulated in table 6. Cumulative percent drug released after 90 min was 76.8 %, 79.8 %, 82.2 % and 94.1 % for SD1 to SD4 respectively and was 38.9 % in 90 minutes for crystalline form of Etravirine. *In vitro* drug release studies revealed that there was the marked increase in the drug release rate of Etravirine from SD1-SD4 when compared to crystalline form of Etravirine. From the *in vitro* drug release profiles, formulation SD4 containing Soluplus (1:2 ratio of drug: Soluplus) was showed higher dissolution rate i.e. 93 % as compared with other formulations. This may be attributed to the increase in drug wettability, conversion to amorphous form and solubilization of the drug using hydrophilic carrier. The graphical representation of drug release profiles of SD1 to SD4 by spray drying method is depicted in fig. 8.

Table 6: *In vitro* dissolution profile of crystalline form of Etravirine, Innovator product and different formulations of Etravirine by spray drying method (SD1, SD2, SD3 and SD4)

Time (min)	Cumulative % drug release					
	Etravirine (Crystalline form)	INTELENCE® 200mg	SD1	SD2	SD3	SD4
0	0	0	0	0	0	0
5	14.9±4.5	22.4±3.9	26.2±4.3	28.2±4.5	25.4±3.2	30.2±3.4
10	20.0±3.7	29.4±2.4	37.3±3.6	37.1±3.2	35.1±2.7	42.1±2.1
20	27.4±3.2	42.8±1.8	48.1±2.7	48.5±2.4	42.2±2.1	53.3±1.8
30	29.2±2.4	52±2.3	55.5±2.1	50.3±2.6	58.5±1.3	68.2±2.3
45	30.3±2.5	66.8±1.6	66.3±1.8	69.4±2.1	63.3±1.8	79.4±1.5
60	34.9±2.2	78.2±2.7	71.4±2.1	77.1±1.8	75.5±1.5	88.2±0.9
90	38.9±1.9	92.8±3.2	76.8±1.5	79.8±0.8	82.2±0.6	93.0±0.6

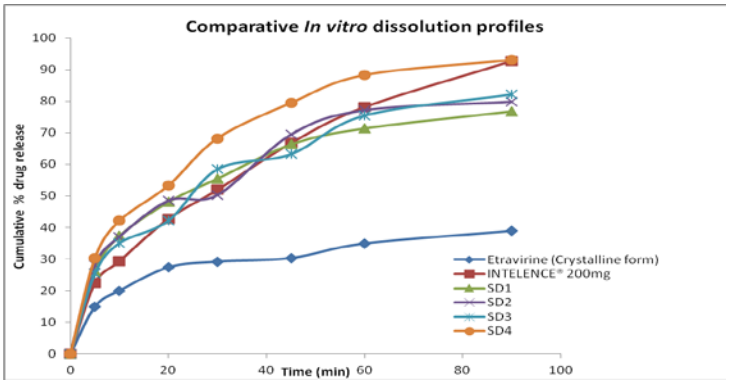


Fig. 8: Comparative *In vitro* dissolution profiles of Etravirine (crystalline), Innovator product and spray drying method formulations (SD1, SD2, SD3 and SD4)

Based on the *in-vitro* dissolution profiles, it was clearly evident that the dissolution profiles of Etravirine in SD4 formulated by spray drying method shown that the rate of drug release was slightly on higher side as compared to that of Innovator product profiles

DISCUSSION

From FT-IR studies, it was found that there was no alteration in the characteristic peaks of in the spray dried powder of SD4, indicates

that there was no interaction between the drug and hydrophilic carrier. On the other hand from the XRD studies, it was evident that the complete transformation of crystalline Etravirine into an amorphous form by Spray drying process. The surface characteristics of spray dried powder of SD4 by SEM showed rough disordered and intact structures indicated that crystalline form of Etravirine has been converted into an amorphous form.

Based on the saturation solubility of drug substance, it was clearly evident that the formulation with Soluplus in the ratio of 1:2 (drug to carrier) which had shown increased solubility by almost 4.0 fold in Purified water and more than 15 folds in pH 6.8 Phosphate buffer as compared to that of the solubility of crystalline form of Etravirine.

In-vitro drug release studies revealed that there was the marked increase in the drug release rate of Etravirine in all four formulations when compared to crystalline form of Etravirine itself. Based on the *in-vitro* dissolution profiles, it was clearly evident that the dissolution profiles of Etravirine in SD4 formulated by spray drying method shown that the rate of drug release was slightly on higher side as compared to that of Innovator product profiles. The increase in solubility and dissolution rate can be explained by polymorphic form conversion from crystalline to an amorphous form.

CONCLUSION

In the present study, the poorly soluble drug substance Etravirine was successfully prepared by Spray drying method. Based on the solubility studies, the solubility of Etravirine after spary drying was increase by more than 4 folds as compared to crystalline form Etravirine in purified water. Based on *in-vitro* drug release profiles of formulation SD4, it was clearly evident that there was a significant increase in the rate of drug release as compared with crystalline form of Etravirine and found to be comparable to that of drug release profiles of corresponding Innovator product (Intelence® 200mg tablet). The results from FT-IR concluded that there was no well defined interaction between Etravirine and carriers. DSC, SEM and XRD showed a change in crystal structure toward an amorphous form of Etravirine. Finally it could be concluded that spray drying of Etravirine along with hydrophilic polymer in a suitable solvent(s) would improves the aqueous solubility, dissolution rate and thereby enhancing its systemic availability.

CONFLICT OF INTERESTS

Declared None

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