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DESIGN AND CHARACTERIZATION OF EFAVIRENZ NANOCRYSTALS FOR SOLUBILITY ENHANCEMENT

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ABSTRACT

Purpose: The main aim of the research work was to develop and evaluate efavirenz nanocrystals to enhance the solubility of poorly soluble efavirenz by nanocrystallization. It is an antiretroviral drug belonging to class II of BCS classification and poorly soluble in aqueous media. **Materials and methods:** There are various techniques and stabilizers used to prepare nanocrystals but in this research work to develop efavirenz nanocrystals using different stabilizer such as PVP K-30, Poloxamer 188, SLS and solvent methanol by antisolvent precipitation method were evaluated. The developed nanocrystals were evaluated for their physicochemical characteristics such as physical appearance, Fourier transform infrared (FTIR), Differential scanning colorimetry (DSC), Scanning electron microscopy (SEM), X-ray powder diffractometry. Drug content, Solubility and In vitro studies were conducted in comparison of nanocrystals to pure efavirenz. **Results and discussion:** There are 12 nanocrystal formulations (K1, K2, K3, K4, P1, P2, P3, P4 and S1, S2, S3, S4) consisting pure drug of efavirenz with PVP K-30, Poloxamer 188 and SLS used as stabilizers in the ratios of 1:1, 1:2, 1:3 and 1:4 respectively. The FTIR spectroscopy was used to confirm compatibility and absence interaction between drug and polymer. DSC and XRD studies of nanocrystals showed degree of crystanallity. Additionally nanocrystals confirmed by scanning electron microscopy. The efavirenz nanocrystal shows uniform drug distribution in the nanocrystals. There is an increase in solubility and the percentage drug release from formulation K4 (optimised efavirenz nanocrystal) was found. **Conclusion:** From the study results, it can be concluded that as compared to pure drug the optimized nanocrystals formulation of has improved solubility. The developed efavirenz nanocrystals found useful to improve solubility.

INTRODUCTION

There are some viruses that are transmitted by bodily fluids i.e. transferred during sex is called as human immunodeficiency virus (HIV). The main action of HIV i.e. retrovirus is seen on immune cells which then progresses the infection to neighbouring cells and thus the immune system becomes weaker and person becomes susceptible to infections. To treat this Type of disease the antiretroviral agent are developed such as Lamivudine (Epivir), Didanosine, Efavirenz (Sustiva), Zidovudine (Retrovir), and Stavudine (Zerit) [1].Efavirenz is a white crystalline powder. The molecular mass of the efavirenz is 315.68. It is practically insoluble in water (<10µg/ml). By its action of non-nucleoside reverse transcriptase inhibitor (NNRTIs) it is acts on type 1 HIV [2].

The drugs having poor solubility may shows biopharmaceutical issues in oral drug delivery like low bioavailability, lack of dose proportionality, high inter-patient variation, retarded onset of action and local irritation. The nanocrystal technology is the field of the Nano science which deals with novel technique to improve the biopharmaceutical issue of poorly soluble drug [3].After oral administration of such nanocrystal drug will contribute to an increased bioavailability, increased adhesiveness to cell membrane and enhance solubility and dissolution velocity as the particle size is in nanometer range [4]. The various advantages of the nanocrystal formulation such as enhanced bioavailability, fast onset of action, high drug load, low incidence of side effects due to the excipients, versatile administration routes such as oral, parenteral, ocular, pulmonary and dermal, no fasted/fed state variation, and an overall improvement of efficiency and safety [5]. In nanospecific formulation the particle size reduction has been a much smarter approach that can be applied for many years. Drug nanocrystals are nanoscopic crystals of parent compound with at least 1 dimension defined as <1000 nm, where 1 nm measuring 1000 millionth of a meter (10^{-9} m) [7]. The drug nanocrystal as its own carrier and typically stabilized with surfactant or polymeric steric stabilizers it means nanocrystal composed of 100 % drug without carriers [6]. In nanocrystal technology the drug nanocrystal can be administered using different administration routes and this can be incorporated into all dosage form such as parenteral, solid, and liquid; fast-melt pulsed release and controlled release oral dosage forms for poorly water soluble compound. In oral administration the patient convenient dosage forms can be produced by transferring the liquid Nanosuspension to solid dosage form, i.e. tablets or pellets or granules containing capsules and is also available in suspension. Other than this, the Nanosuspension can be injected parenterally and by intravenous injection which may lead to 100% bioavailability because of their small size [7].In this field of biomedical sciences, such as drug therapy, diagnostics and imaging the nanotechnology is expected to facilitate revolutionary innovations. Now and in the years to come the drug delivery and clinical applications of nanotechnology is one of the key factors for modern drug therapy. In this research work, approaches are made to enhance the solubility and dissolution rate by formulating nanocrystals [8].

MATERIALS AND METHODS

Materials

Efavirenz drug was gift sample from Aurobindo pharma limited (Hyderabad, Andhra pradesh, India). Poloxamer 188 and sodium lauryl sulphate were supplied by also Aurobindopharma limited (Hyderabad, Andhra pradesh, India). All other solvents and reagents in this research work were analytical grade. The materials which are received are used directly without any further purification.

CHARACTERIZATION OF PURE EFAVIRENZ

1. **Determination of organoleptic properties of API**

   Physical appearance, colour and odour of efavirenz were determined.

2. **Determination of solubility profile of drug**

   By adding a pinch of drug in the solvents such as water, methanol, ethanol and dichloromethane etc. the solubility of efavirenz was checked.

3. **Determination of melting point of drug**

   It was determined by capillary tube method.

4. **Identification of drug**

UV Spectra: At the concentration of 10 µg/ml of methanol solvent used to recorded UV spectrum of efavirenz. At the wavelength of 247 nm the λmax values of bands lie.

Selection of solvent: For spectrophotometric analysis of efavirenz, methanol was selected as ideal solvent.

PREPARATION OF EFAVIRENZ NANOCRYSTALS

The preparation process involves following steps [9, 10]: Preparation of drug solution;
Addition of drug solution to miscible antisolvent;

Preparation of drug solution: Preparation of drug solution by using organic solvent: Efavirenz was soluble in methanol so, drug solution was prepared by using organic solvent methanol.

Addition of drug solution to miscible anti solvent:

Addition of Efavirenz drug solution in water: Above drug solution was added in sufficient amount of water containing polymeric stabilizers (PVP K30, Poloxamer, and SLS) with continuous stirring. Instantly, from the antisolvent the particles are precipitated and formed milk like suspension which was then filtered and dried.

CHARACTERIZATION OF NANOCRYSTALS

Solubility study [11, 12, and 13]:

The solubility of EFZ and nanocrystal was carried out by taking amount of 10 mg of pure drug and prepared nanocrystals were separately in to purified water, to obtained saturated solution. By using thermostatically controlled rotary shaker the sealed flasks were agitated for 24 hrs at 37ºC. Then the aliquot was filter by using 0.45 µm membrane filter. Then the filtrate was diluted and analysed UV visible at wavelength 246 nm.

Drug content [14, 15, and 16]: The 10 mg of nanocrystal powder dissolve in 5 ml methanol and by using phosphate buffer pH 7.4 the volume was made up to mark in 100 ml volumetric flask. Then the above solution was diluted and analysed by spectrophotometrically at 246nm. By using calibration curve the EFZ content in nanocrystals was calculated.

Scanning electron microscopy (SEM): The optimized formulation was examined for its surface characteristics and compared to efavirenz pure drug by using scanning electron microscopy (FEI NOVA Nano FESEM 650). The detailed particle structural characterization was conducted at 15 KV acceleration voltage. The mean particle size was calculated by the diameters of individual nanocrystals of the SEM images.

Fourier transfer infrared spectroscopy (FTIR): To check compatibility of drug with the excipients the FT-IR spectra of pure drug (EFZ) and nanocrystals of drug were carried out. The samples were mixed with KBr powder and pellets were then scanned using FT-IR spectrophotometer. The wavelength ranged from 400-4000 cm^{-1}. Compare the FT-IR spectra of mixture with the FT-IR spectra of pure drug.

Differential scanning calorimeters (DSC): The DSC studies were performed using a METTLER DSC instrument to evaluate thermal properties of the efavirenz pure drug and drug nanocrystals. To analyse 1.4000 mg amount of product and be placed in crimped aluminium sealed 40 ul pans. At a scanning rate of 10 K/min heat runs for each sample has been set from 25 to 300ºC, under dry nitrogen flow 20 ml/min.

Powder X-ray diffraction (PXRD): The optimized nanocrystals formulation was recorded for the crystalline properties and compared to efavirenz pure drug. Standard runs using a 40 mA current, 40 KV voltage and over range of 2ɵ angle was used between 1^0 to 75^0 at a scanning rate and step size of 0.02^0 /min.

***In vitro* release study [17, 18, 19, and 20]:** By using dialysis bag diffusion technique the in vitro release of EFZ from nanocrystals was evaluated. The pure drug and prepared nanocrystals were added in glass beaker containing methanolic phosphate buffer saline (pH 6.4, 50% v/v) and it was used as a diffusion medium. The beakers were placed in magnetic stirrers and stirred with magnetic beads. Aliquots were withdrawn periodically and replaced with the same volume of fresh media and filtered, diluted and were finally analysed by UV-visible spectrophotometrically at 246 nm.

RESULTS AND DISCUSSION

Solubility study: As compare to pure efavirenz (0.017) the formulation of K4, F3 and S4 showed highest solubility in water (0.077, 0.069 and 0.061 respectively). Significantly, other formulations of efavirenz are also shows increase in the solubility of efavirenz in water.

Drug content: The K4, F3 and S4 showed highest uniform distribution of drug in the nanocrystals (80.41, 72.33 and 69.16 % respectively) it can be determined by drug

content analysis and other formulations of efavirenz are also shows better dissolution of drug.

Scanning electron microscopy (SEM): The surface morphology of nanocrystals was investigated by scanning electron microscopy. The SEM images of Efavirenz nanocrystals are shown in Fig. 1, 2, and 3. From these images it was observed that the nanocrystals appear in irregular shape.

Fourier transfer infrared spectroscopy (FTIR): To check the compatibility between drug and excipients the FTIR spectroscopy was carried out. The pure drug showed characteristic peaks at 3314.19 cm^{-1} (N-H stretching), 2249.60 cm^{-1} (C=C Alkyne), 1744.15 cm^{-1} (C=O of ester), 1601.77 cm^{-1} (C=O of Amide), 1494.79, 1315.79 cm^{-1} (C-F), 1096.59 cm^{-1} (C-Cl). All these noted peaks of EFZ were present in EFZ in combination with excipients. It is clearly indicates in EFZ nanocrystals without losing its characteristics the drug has retained its identity. When nanocrystals prepared using PVP, Poloxamer 188 and SLS the IR peak shows the absence of interaction between drug and excipients. In the FTIR spectra of nanocrystals the new bands found may be due to the poloxamer and SLS. The IR spectrum of pure EFZ and EFZ nanocrystals of K4, F3, and S4 batch are shown in fig. 4, 5, 6, 7.

Powder X-ray diffraction (PXRD): To know the nature of the compound powder X-ray diffraction analysis was performed. The powder XRD diffractograms are shown in the fig. 8, 9, 10, and 11. The pure efavirenz drug shows characteristics crystalline peaks at 14.572, 7.225, and 6.692. The powder XRD diffractogram of efavirenz nanocrystal of K4 batch shows characteristics crystalline peaks at 14.525, 7.213 and 6.692. Efavirenz nanocrystal F3 batch shows characteristics crystalline peaks at 14.525, 7.13 and 6.692 and efavirenz nanocrystal S4 batch shows characteristics crystalline peaks at 14.525, 7.213 and 6.692 with less intensity.

Differential scanning colorimetry: A DSC study was carried out to explore the physical changes that occurred in the drug after processing in to nanocrystals. The efavirenz pure drug exhibited a single, large and sharp endothermic peak at 138^0C indicated its melting point shown in fig. 12. The DSC thermogram of optimized nanocrystal formulations K4, F3 and S4 showed an endothermic peak at 137^0C, 138^0C and 136^0C respectively ascribed to the melting point of EFZ, indicated the slight change in crystallanity due to change in melting point shown in fig.13, 14, 15. It should be conclude that reduction in melting temperature could increase the dissolution rate.

***In vitro* release study**: With pure drug efavirenz the in vitro release profiles of all nanocrystals were compared. For 2 hours the release studies were carried out and results obtained are tabulated in table. The pure efavirenz drug release was found to be 34.20. the drug release were found to be 43.48, 58.07, 63.46, 78.46, 38.07, 49.23, 68.07, 64.61, 35.00, 44.23, 56.53, 67.69 for formulation of K1, K2,K3, K4, F1, F2, F3, F4, S1, S2, S3, S4 batch respectively. From it was were concluded that the as compared to pure efavirenz drug the K4 batch of PVP K-30, F3 batch of poloxamer f-68, and S4 batch of SLS shows increase in the efavirenz release study.

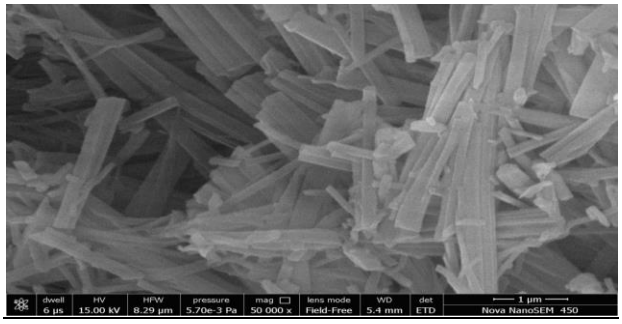

Fig. 1: SEM of K4 batch

Table 1: Formulation of Efavirenz Nanocrystals.

Formulation code	Drug: Polymer ratio	Solvent
K1	1:1	Methanol
K2	1:2	Methanol
K3	1:3	Methanol
K4	1:4	Methanol
F1	1:1	Methanol
F2	1:2	Methanol
F3	1:3	Methanol
F4	1:4	Methanol
S1	1:1	Methanol
S2	1:2	Methanol
S3	1:3	Methanol
S4	1:4	methanol

Table 2: Solubility of Efavirenz Nanocrystals.

Formulation code	Solubility (µg/ml)
Pure Efavirenz	0.017
K1	0.049
K2	0.055
K3	0.064
K4	0.077
F1	0.043
F2	0.048
F3	0.069
F4	0.057
S1	0.032
S2	0.039
S3	0.049
S4	0.061

Table 3: Drug Content of Efavirenz nanocrystals.

Formulation Code	Drug Content (%)
K1	46.58
K2	58.16
K3	64.83
K4	80.41
F1	41.00
F2	50.16
F3	72.33
F4	65.16
S1	38.83
S2	49.25
S3	60.08
S4	69.16

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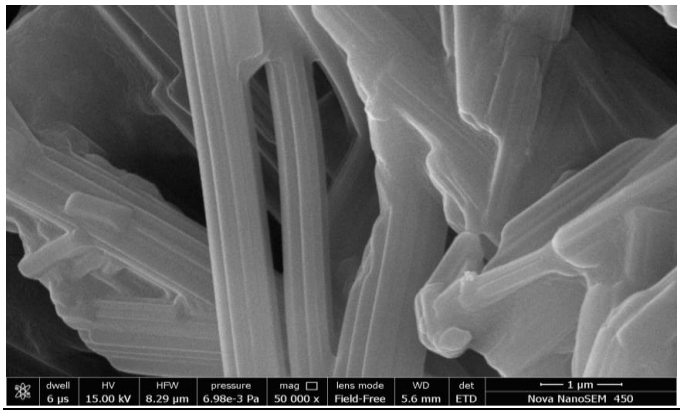

Fig. 2: SEM of F3 batch.

Fig. 3: SEM of S4 batch.

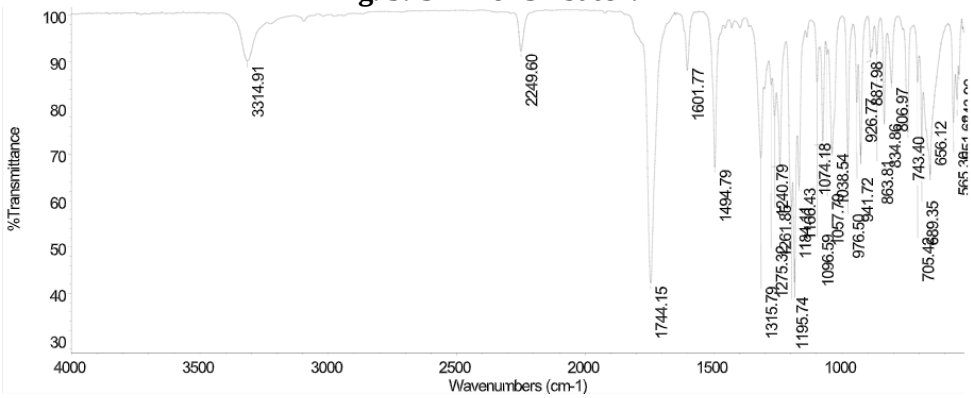

Fig.4: FT-IR spectrum of pure drug efavirenz

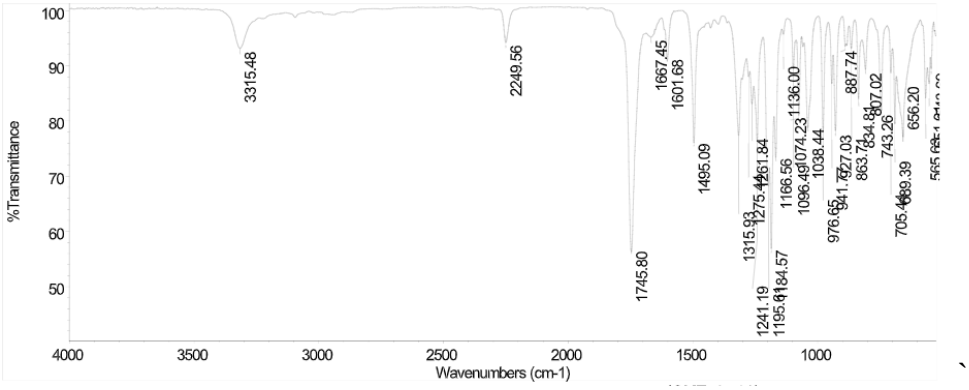

Fig. 5: FT-IR spectrum of K4 batch

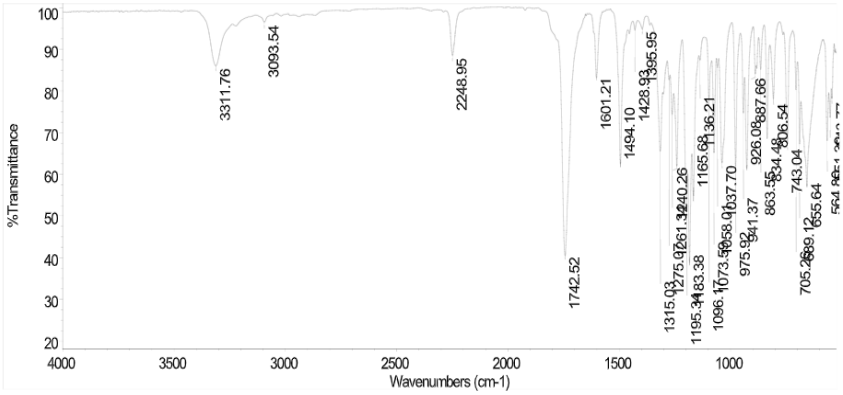

Fig.6: FT-IR Spectrum of F3 batch

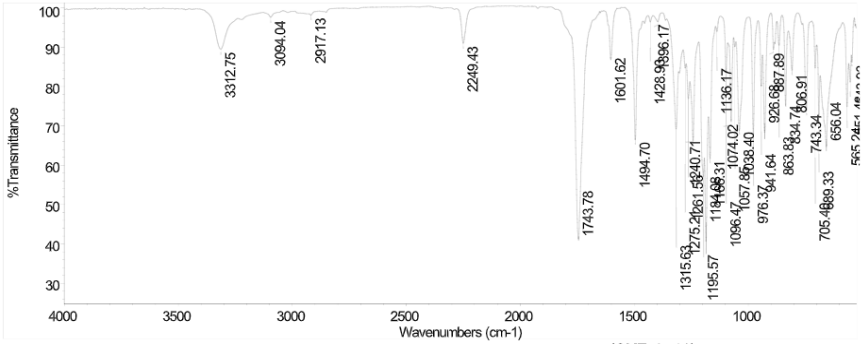

Fig.7:FT-IR spectrum of S4 batch

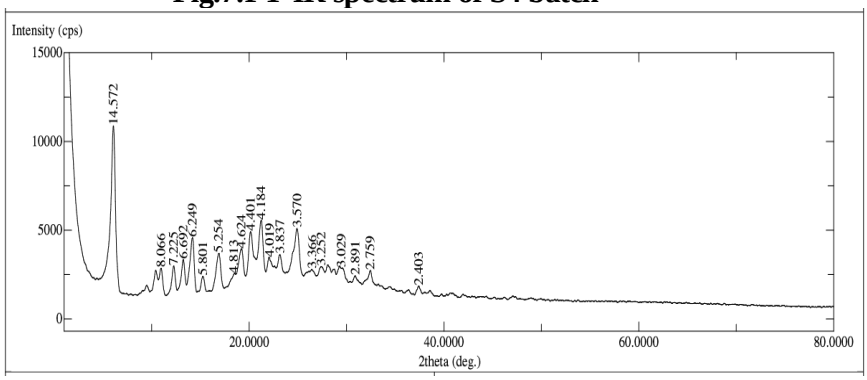

Fig.8: P-XRD of Pure drug Efavirenz

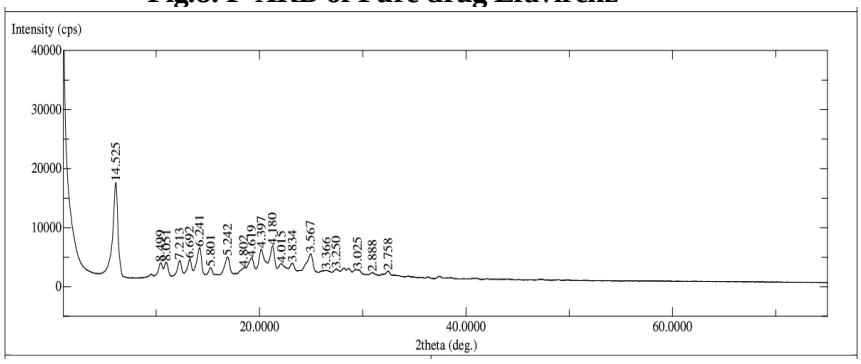

Fig.9: P-XRD of K4 batch

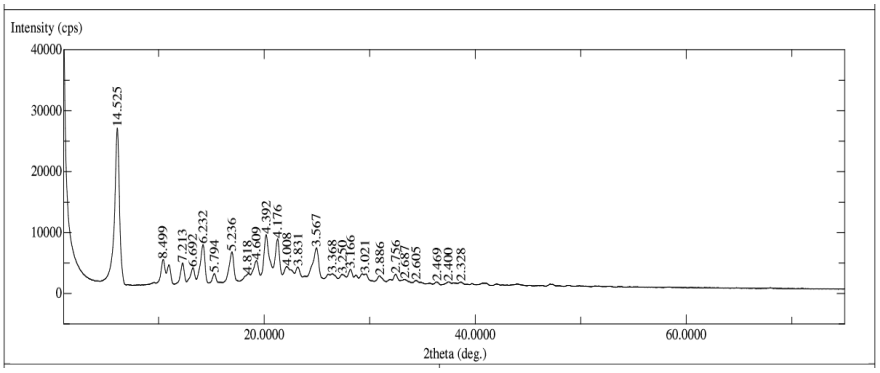

Fig.10: P-XRD of F3 batch

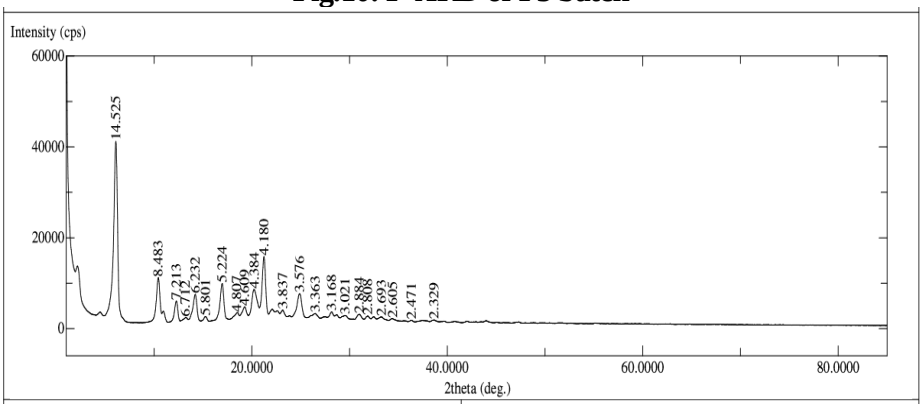

Fig.11: P-XRD of S4 batch

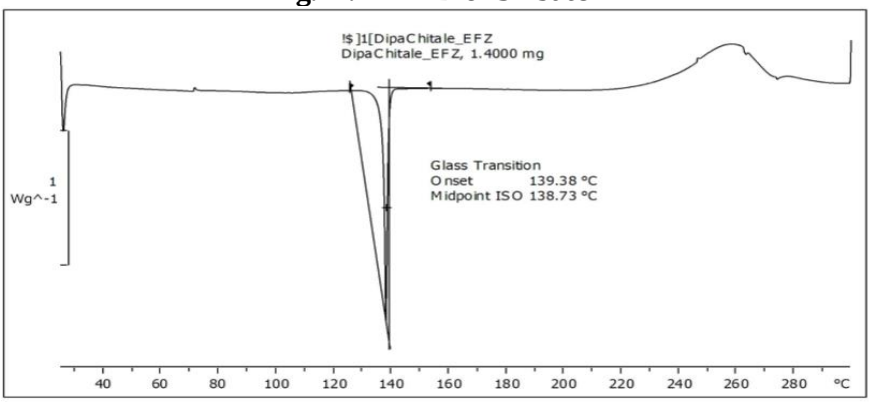

Fig.12: DSC thermogram of pure EFZ drug

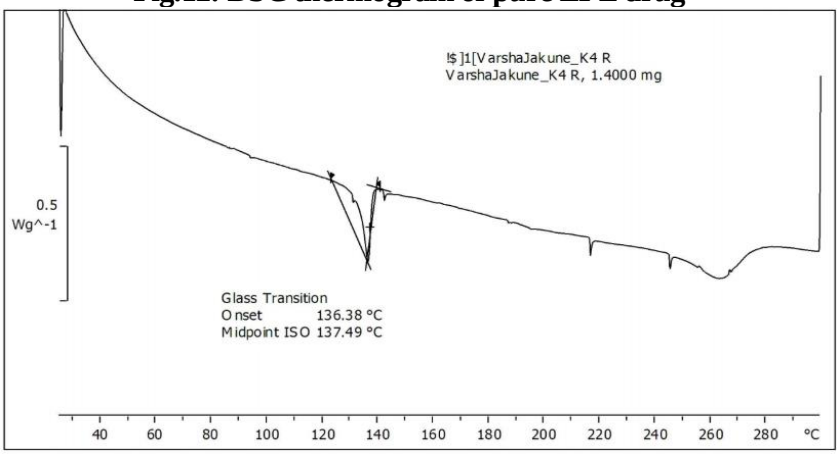

Fig.13: DSC thermogram of K4 batch

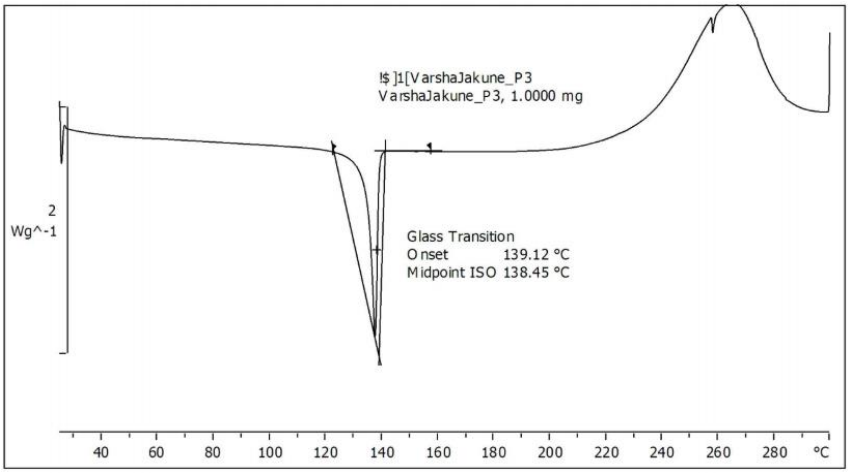

Fig.14: DSC thermogram of F3 batch

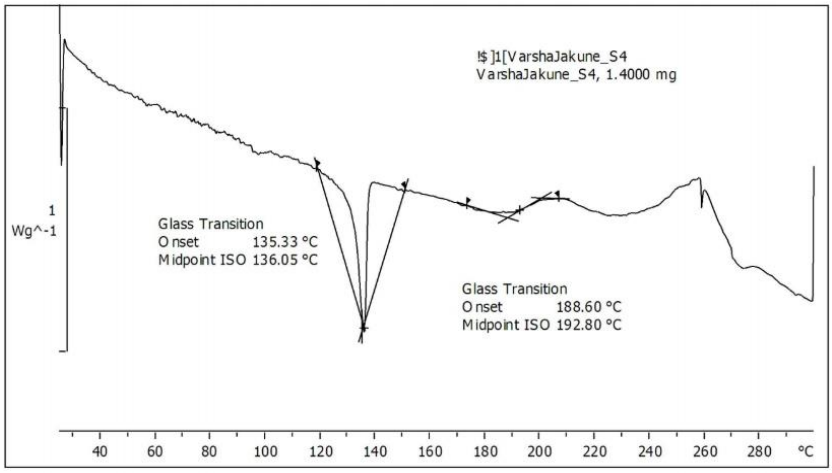

Fig.15: DSC thermogram of S4 batch

Table 4: *In vitro* dissolution of efavirenz pure drug and nanocrystals.

Time (min)	Cumulative % release of Efavirenz Nanocrystals												
	Pure EFZ	K1	K2	K3	K4	F1	F2	F3	F4	S1	S2	S3	S4
0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	12.69	23.07	28.07	35.38	39.61	22.30	24.61	37.69	36.15	20.76	21.92	35.76	50.38
30	18.46	26.53	31.15	41.53	53.84	27.69	30.00	47.30	48.07	23.46	23.84	39.23	53.46
45	24.23	33.46	38.84	46.92	65.38	30.38	36.15	50.00	48.84	25.76	27.69	42.30	56.92
60	26.92	36.15	46.53	52.69	69.61	31.53	42.69	61.15	60.38	28.46	33.84	48.07	61.92
90	29.61	40.38	51.53	60.76	74.23	33.46	45.76	65.00	61.92	30.00	39.23	53.07	65.38
120	34.23	43.48	58.07	63.46	78.46	38.07	49.23	68.07	64.61	35.00	44.23	56.53	67.69

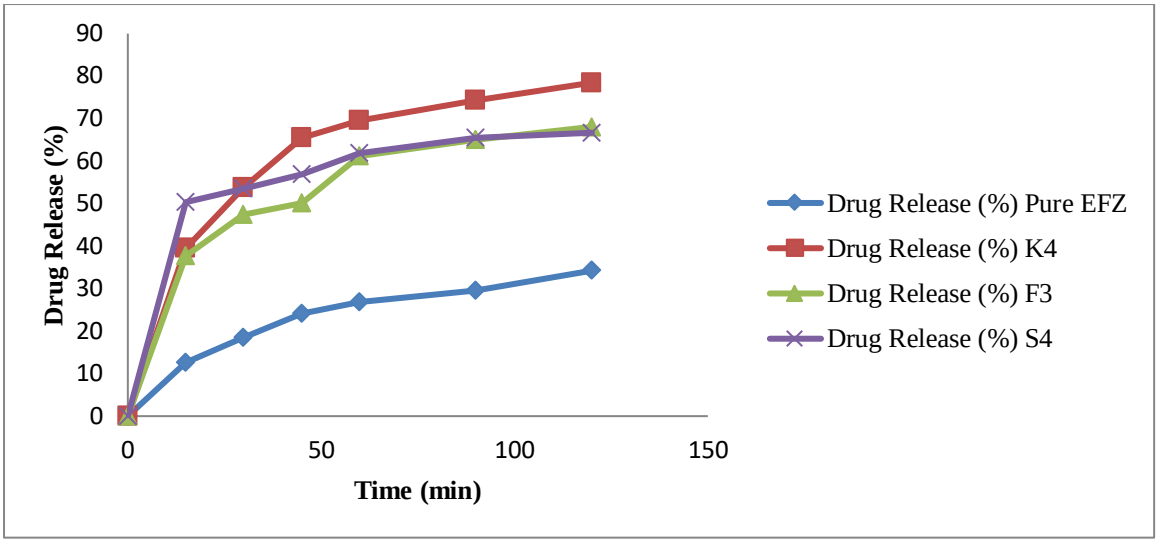

Fig. 16: *In- Vitro* drug release

CONCLUSION

The purpose of the research work was to enhance the solubility of the drug by preparing Efavirenz nanocrystals. The nanocrystals were prepared by anti-solvent precipitation method by using polymers such as PVP, Poloxamer 188 and SLS in different concentrations and methanol was used as a solvent. Total 12 batches of nanocrystals were prepared. By FTIR studies, it was proved that there were no interaction between the drug and the polymer. The Efavirenz nanocrystals shows uniform drug distribution and it was found that K4 batch shows 80.41 %, F3 batch shows 77.33 % and S4 batch shows 69.16 % uniform drug distribution. There was an increase in solubility of efavirenz drug. The percentage drug release from formulation K4, F3 and S4 was found to be 78.46 %, 68.07 %, and 67.69 % respectively. A DSC study shows a slight change in the crystallanity due to change in melting point. It was proved that reduction in melting temperature could increase the dissolution rate. P-XRD graphs of nanocrystals show their crystalline nature. The SEM analysis of nanocrystal shows the surface morphology of the particles.

It was concluded that the EFZ nanocrystals produced by antisolvent precipitation method using PVP K-30, Poloxamer 188 and SLS as polymers showed increase in solubility and release of drug.

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