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Research Article

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Solubility and dissolution rate enhancement of efavirenz by inclusion complexation and liquid anti-solvent precipitation technique

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ABSTRACT

The liquid anti-solvent (LAS) precipitation process for production of ultra-fine particles has been widely employed for enhancing the solubility and dissolution rate of poorly water soluble drugs. In LAS process, precipitation of solute is achieved by decreasing the solvent power for the solute dissolved in a solution. A non-solvent or organic solvent for the drug is added to a polar or aqueous anti-solvent. The current research work endeavors to provide an account on the application of LAS for precipitation and stabilization of ultrafine micro or nanoparticles of poorly water soluble drug, Efavirenz, and comparison of its aqueous solubility and dissolution rate with β-cyclodextrin and hydroxypropyl-β-cyclodextrin inclusion complexes of the drug. Besides, the key aspects like controlling the particle size and size distribution, and stabilization of ultrafine particles using polymers has been studied. Applications of Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and powdered X-ray diffraction (PXRD) techniques in physico-chemical characterization and evaluating the eventual transformation of solid state characteristics of the drug from crystalline to amorphous state has also been conducted. The findings of the work corroborated suggested the suitability of prepared polymeric drug micro or nano-crystals of Efavirenz in enhancing its solubility.

Key words: liquid anti-solvent (LAS) precipitation, efavirenz, inclusion complexation, dissolution, physicochemical characterization.

INTRODUCTION

In modern drug development, high throughput screening techniques aid in identifying new compounds that are characterized by high lipophilicity, low aqueous solubility and poor dissolution. Thus, improving the solubility of poorly soluble drugs should increase the concentration of dissolved drug in the gastrointestinal (GI) tract and thus increase their bioavailability. A higher energetic state of the compound, that is, the amorphous state, can increase drug solubility and improve bioavailability. Due to their simple manufacture, solid dispersions are a popular means for enhancing solubility and bioavailability of BCS class II drugs [1].

Several strategies and formulation approaches have been employed to overcome their limitations, such as usage of complexing agents such as cyclodextrins; use of suitable polymorphic forms; use of co-solvents, micellar solutions, and lipid-based systems for lipophilic drugs [2].

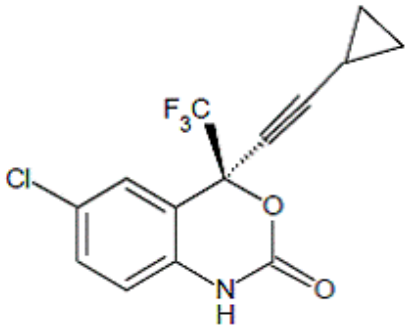
The central objective of a delivery system is the release of therapeutics at the desired anatomical site and the maintenance of the drug concentration within the therapeutic range for a desired duration. One approach for drug

delivery is to encapsulate the drug molecule inside a macrocyclic host molecule, traditionally a cyclodextrin, thereby forming discrete host–guest complexes, in which the drug molecule is protected from the aqueous environment [3]. CDs are cyclic oligosaccharides with hydrophilic outer surface and a somewhat lipophilic central cavity. In aqueous solutions CDs are able to solubilize lipophilic drugs through formation of hydrophilic inclusion complexes. These are attractive building blocks for various types of drug delivery systems due to their favorable toxicological profile and their inherent ability to partly or completely host biologically active molecules (e.g., drugs), and to protect them from the external environment. The high affinity of CDs for certain drug molecules is passed on to the carrier systems which give them with a particular drug release mechanism thus helps to improve solubility in aqueous media, dissolution rate, chemical stability and bioavailability of various drugs has been extensively investigated in recent years [4-6].

Micronization or nanonization produces ultrafine powder either in the micron or nanometer size range with an exponential increase in the interfacial area of contact between the dissolving particles and the dissolution medium thereby enhancing dissolution rate of the drug.

Top-down methods involve breakdown of coarse particles to the required size range while bottom-up methods involve building particles from solutions by addition of anti-solvent. Mixing a solution with an anti-solvent generates super saturation that induces nucleation and simultaneous growth by condensation and coagulation. The drug must be soluble in the solvent but practically not soluble in the anti-solvent. The solvent and anti-solvent must also be miscible at the operating conditions. Mixing is a critical factor for controlling the final particle size and size distribution [7, 8].

The aim of the present study was to prepare nanoparticles of Efavirenz by the liquid anti-solvent (LAS) precipitation technique. Efavirenz is (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1benzoxazin-2-one. Practically insoluble in water (<10µg/ml), belongs to BCS Class II drugs. It is a Non-Nucleoside Reverse Transcriptase Inhibitor, widely prescribed for the treatment of human immunodeficiency virus type 1 infection. Its pKa and melting point, determined experimentally, were found to be 10.2 and 161-166 ^{0}C, respectively. The structure of Efavirenz is shown in **Figure 1.**



EXPERIMENTAL SECTION

Materials

Efavirenz was provided by Aurobindo Pharmaceuticals, Hyderabad. β- Cyclodextrin and Hydroxypropyl-β-Cyclodextrin were from Glenmark Ltd., Mumbai. All chemical and reagents used are of analytical grade range.

Methodology

Analytical Method

Linear plot for Efavirenz is obtained at absorbance maxima for the drug at 250 nm with a correlation coefficient of 0.9993.

Preparation of Inclusion Complexes

Inclusion complexes of Efavirenz with β-CD or HP β-CD were prepared by kneading technique in the ratios of 1:0.25, 1:0.5, 1:1, 1:2 and 1:3 (w/w Drug : Carrier). Inclusion complexes of drug with β-CD or HP β-CD were prepared by kneading using 8:2 (v/v) mixture of water - ethanol and dried at 45 to 50 ^{0}C. All the complexes were

prepared in triplicate and were sieved through 60 mesh sieve and stored over anhydrous calcium chloride in a desiccator.

ICs by Physical mixing in the same drug to carrier ratio were prepared by simple mixing under trituration using a ceramic mortar [9].

Anti-solvent Precipitating Technique

Weighed quantity of drug was dissolved in ethanol in a beaker. The drug solution was then added to a beaker containing water under sonication using an ultra sonicator, using syringe with a needle (No. 26). Then the beaker content was dried on water bath to evaporate water. The obtained precipitate was then passed through 44 mesh sieve and stored in desiccators until further use.

Solubility studies

The phase solubility or saturation solubility study was conducted by adding excess amount drug or formulations to vial containing 20 mL of distilled water. The system was agitated on a water bath shaker for 48 hrs and filtered. The filtrate was analyzed using UV-Visible spectrophotometer at 250 nm. The studies were carried out in triplicate and the average value was noted. The Gibbs free energy of transfer (ΔG_{tr}^{0}) of Efavirenz from pure water to the aqueous solution of carriers was calculated using following Eq. 1.

$$\Delta G_{tr}^{0} = -2.303\ RT \log S_0/S_s \qquad \text{Eq. ------ (1)}$$

Where S_0/S = the ratio of molar solubility of drug in aqueous solutions of carriers to that of pure water. Gibbs' free energy ΔG_{tr}^{0} (J/mol) [9].

In Vitro Drug Release Studies

Dissolution studies of Efavirenz, in pure form, ICs and precipitates by anti solvent technique were performed by using the U.S. Pharmacopoeia (USP) model digital dissolution test apparatus Type-2 (Lab India, Mumbai) at the paddle rotation speed of 50 rpm using 900 mL distilled water containing 0.5 % w/v of SLS as dissolution medium at 37±0.5 $^{\circ}$C. The preparations equivalent to 100 mg of the Efavirenz was weighed using a digital balance (Sartorius) and added to the dissolution medium. At the specified times, 10 mL samples were withdrawn by using syringe filter (0.45µm) (Sepyrane, Mumbai) and then quantitated for the drug release by measuring the absorbance at 290 nm using the UV-Visible spectrophotometer (Agilent Cary 60) and volume was adjusted by fresh medium maintained at 37 $^{\circ}$C after each sampling to maintain its constant volume throughout the test. Dissolution studies were performed in triplicate (n=3), calculated mean values of cumulative drug release and data were used while plotting the release curves [10].

Fourier-Transform Infrared Spectroscopy

The FTIR spectra were obtained by using an FTIR spectrometer (Shimazdu, Japan). The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:100 (Sample: KBr) ratio respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Scans were obtained at a resolution of 2 cm^{-1}, from 4000 to 400 cm^{-1}.

Differential Scanning Calorimetry

Thermal analysis was performed on a DSC- 6100 (Seiko Instruments, Japan). All accurately weighed samples (2 mg of Efavirenz or its equivalent) were placed in sealed aluminium pans, before heating under nitrogen flow (20 mL/min) at a scanning rate of 30 ^{0}C min^{-1} from 30 to 300 $^{\circ}$C. An empty aluminium pan was used as reference.

X-ray Diffraction

The X-ray powder diffraction patterns were recorded at room temperature using a PW1710 X-ray diffractometer (Philips, Holland) with Cu as anode material and graphite monochromatic, operated at a voltage of 35 kV, current 20 mA. The samples were analyzed in the 2θ angle range of 5°–70° and the process parameters were set as: scan step size of 0.02° (2θ), scan step time of 0.5 s.

RESULTS AND DISCUSSION

Solubility Studies

Solubility experiments showed that the concentration of Efavirenz in water is notably affected by the presence of carriers. Aqueous solubility of Efavirenz was noted to be 13 µg /mL; whereas the reported value of its aqueous solubility is < 10 µg/mL. With increase in concentration of β-CD and HP β-CD the solubility of Efavirenz found increased significantly. It has been found that hydrophilic carriers mainly interact with drug molecules by electrostatic bonds (ion-to-ion, ion-to-dipole, and dipole-to-dipole bonds), even though other types of forces, such as van der Waals forces and hydrogen bonds, can frequently play a role in the drug-carrier interaction. The values of Gibbs free energy associated with the aqueous solubility of Efavirenz in presence of carrier, the ΔG_{tr}^{o} values were all negative for carriers at various concentrations indicating the spontaneous nature of the drug solubilization. The values decreased by increasing carrier concentration, demonstrates that the reaction became more favourable as concentration of carrier increased (Table 1).

Table 1: Gibbs' free energy ΔG_{tr}^{0} (J/mol) values from Solubility studies

Polymer Concentration	β – CD	HP β-CD	HPMC
2%	-3442.63	-3260.42	-2332.73
4%	-4046.39	-4486.22	-3922.78
6%	-4092.09	-4697.56	-4291.38

***In Vitro* Dissolution Data**

Dissolution Studies of Pure Efavirenz

The maximum dissolution for Efavirenz was found to be 43.96% after 60 minute and the dissolution profile is shown in **Figure 2.**

The drug release study from inclusion complexes by physical mixing and kneading with β –CD is given in **Table 2**

Table 2: Drug release from inclusion complexes by physical mixing and kneading with β –CD

Time (min)	% DRUG RELEASE BY PHYSICAL MIXING					% DRUG RELEASE BY KNEADING METHOD				
	1:0.25	1:0.5	1:1	1:2	1:3	1:0.25	1:0.5	1:1	1:2	1:3
10	31.46	35.06	39.64	44.14	26.66	40.76	43.74	40.38	44.09	14.89
15	36.74	38.79	40.38	44.48	27.93	44.29	44.48	44.44	50.10	25.24
30	45.28	43.11	52.20	52.34	30.37	47.55	48.91	53.43	56.75	24.52
45	51.70	53.03	56.76	60.04	31.00	51.41	55.51	64.12	65.93	25.86
60	56.76	60.25	64.13	66.15	32.86	62.26	64.17	67.87	68.82	27.69

The drug release study from inclusion complexes by physical mixing and kneading with HP β –CD is given in **Table 3**

Table 3: Drug release from inclusion complexes by physical mixing and kneading with HP β- CD

Time (min)	%DRUG RELEASE BY PHYSICAL MIXING					%DRUG RELEASE BY KNEADING METHOD				
	1:0.25	1:0.5	1:1	1:2	1:3	1:0.25	1:0.5	1:1	1:2	1:3
10	35.55	39.30	40.39	44.55	30.82	43.59	44.10	4.4845	45.69	13.14
15	44.13	40.35	43.2450	48.57	31.26	44.40	45.80	47.06	51.77	15.20
30	47.01	48.39	53.8445	56.39	33.44	48.11	50.85	55.49	59.61	17.25
45	53.84	56.84	61.6336	66.16	34.79	55.89	60.96	65.02	69.87	17.39
60	63.71	64.93	67.80	72.27	37.11	64.73	70.23	73.62	82.84	17.95

Table 4: Drug Release Studies of Efavirenz from Precipitates by Anti-solvent Method

Time (min)	% Drug release (in presence of Different concentrations of HPMC)			
	1:0	1:0.125	1:0.25	1:0.5
10	44.4518	62.5090	42.8645	22.9909
15	49.3036	70.2204	43.0895	31.176
30	60.3654	77.0727	52.3431	35.9631
45	65.6059	82.8572	61.5313	43.9977
60	70.6663	87.7663	69.1609	57.6368

The drug release study from preparation by liquid anti solvent precipitation technique in presence of different concentration of HPMC is given in **Table 4**

Figure 2: Release profile of Efavirenz and its optimized formulations with different carriers

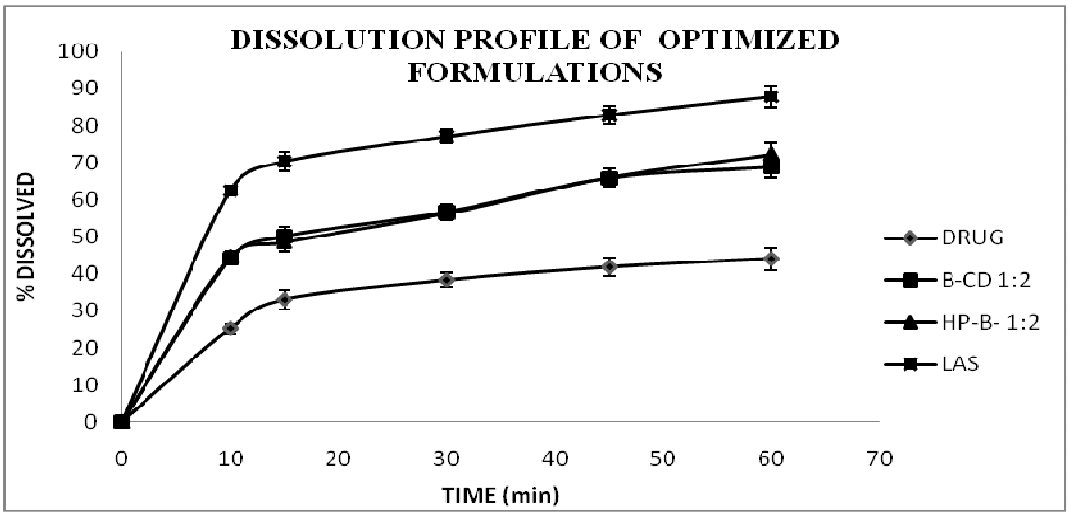
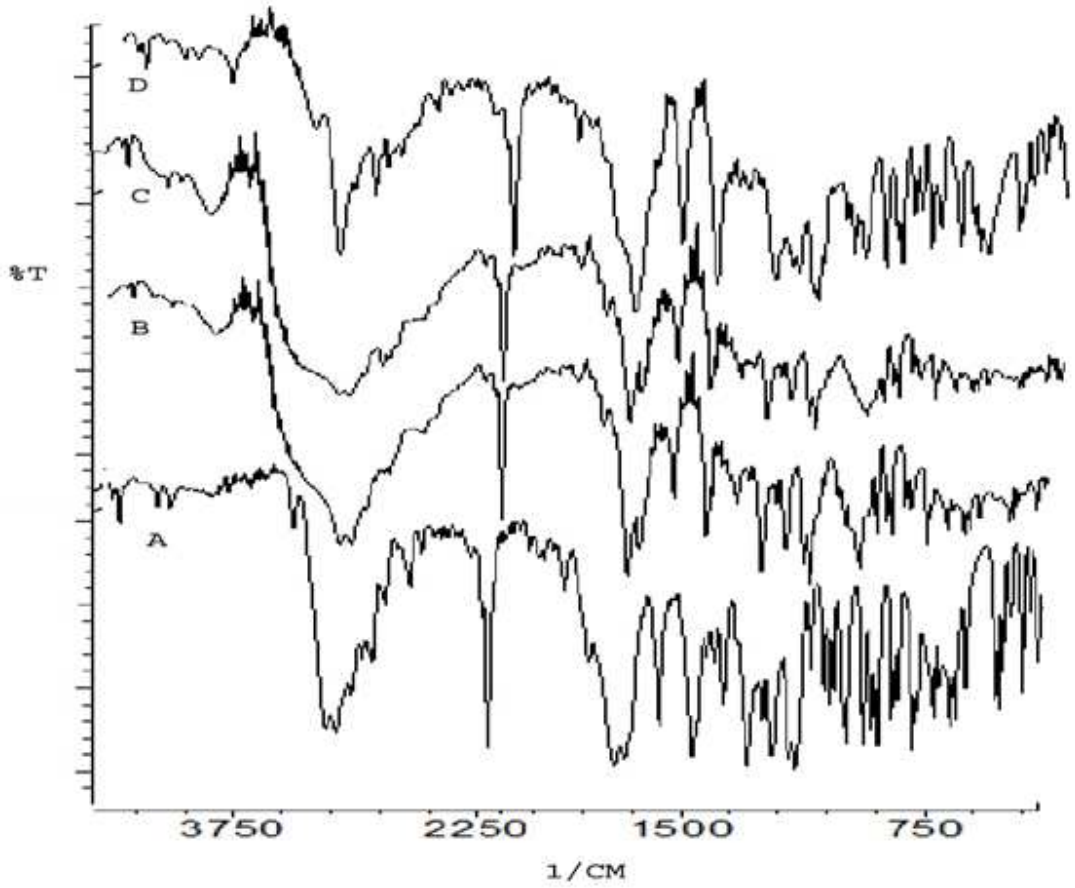


Figure 3: (A) IR spectrum overlay for Efavirenz, (B) Efavirenz with β CD 1:2 ratio (C) Efavirenz with HP β CD 1:2 ratio and (D) Efavirenz drug precipitated by LAS technique in presence of HPMC at 0.125%



The drug release profile of Efavirenz and its inclusion complexes and Antisolvent precipitation technique at their optimum ratio (1:2 rations for both β CD and HP β CD by kneading technique and anti solvent precipitation with 0.125% HPMC) is shown under **Figure 2.**

Fourier Transform Infrared Spectroscopy (FTIR):

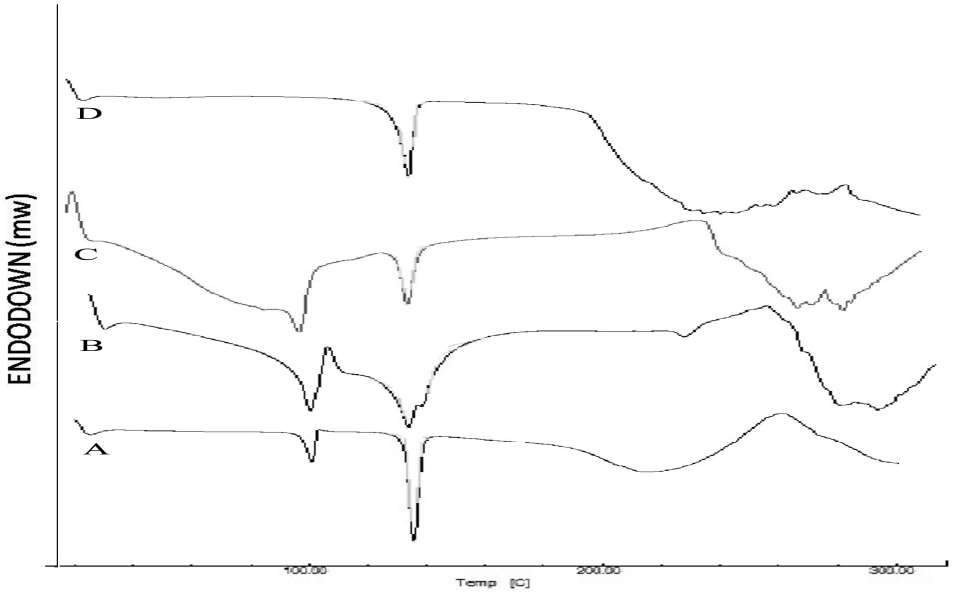
The IR spectrum overlay for Efavirenz (A), Efavirenz with β CD 1:2 ratio (B), Efavirenz with HP β CD 1:2 ratio (C) and Efavirenz drug precipitated by LAS technique in presence of HPMC at 0.125% (D) is given under **Figure 3.**
From the IR interpretation the characteristic frequencies in FTIR spectrum of Efavirenz is given under **Table 5**

Peak value	Inference
3180 cm-1	N-H stretching vibration
3052 cm-1	Aromatic C-H stretching vibration
2841 cm-1	Aliphatic C-H stretching vibration
1691 cm-1	C=O
1589 cm-1	C=C
1217 cm-1	C-F

Differential Scanning Calorimetry

The DSC thermogram overlay for Efavirenz (A), Efavirenz with β CD 1:2 ratio (B), Efavirenz with HP β CD 1:2 ratio (C) and Efavirenz drug precipitated by LAS technique in presence of HPMC at 0.125% (D) is given under **Figure 4.**

Figure 4: (A) DSC thermogram overlay for Efavirenz, (B) Efavirenz with β CD 1:2 ratio, (C) Efavirenz with HP β CD 1:2 ratio and (D) Efavirenz drug precipitated by LAS technique in presence of HPMC at 0.125%



X-ray Diffraction

The X-ray powder diffraction pattern for the optimized preparation by LAS precipitation technique which showed the satisfactory solubility enhancement of Efavirenz is only given for XRD under **Figure 5**

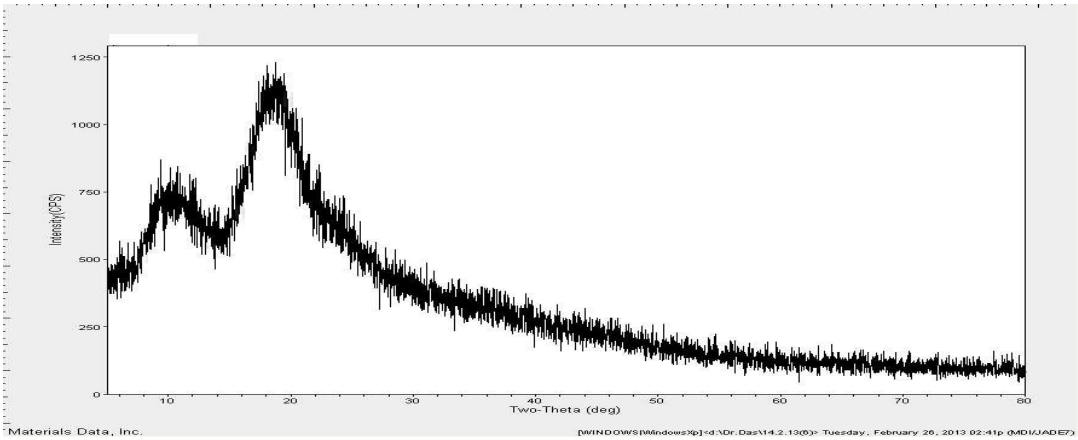


Figure 5: XRD of Efavirenz with HPMC (LAS)

DISCUSSION

The increase in negative values of Gibbs free energy of transfer from water to an aqueous solution of hydrophilic carriers indicated the spontaneity of drug solubilization. The solubility and dissolution rate of Efavirenz can been enhanced by the Antisolvent precipitation technique of Efavirenz in water containing HPMC. The solubility improvement of precipitated drug may be contributed due to reduction of particle size i.e., micro or nano sizing, reduction of aggregation of the drug due to presence of HPMC, absence of crystallinity, increased dispersibility and alteration of surface properties of the drug in its solid dispersion. [11]

Among the various ratios, Efavirenz precipitates obtained in presence of HPMC at 1:0.125w/w prepared by anti-solvent technique showed satisfactory solubility enhancement i.e., nearly 90 % in 60 minutes, where as for pure drug of Efavirenz it is only 43 % in 60 minutes. Among various ratios of Efavirenz: HP β CD prepared by kneading method, ratio at 1:2w/w showed better enhancement of solubility i.e.,75 % in 60 minutes and it is observed that increased polymer weight ratio has no further significant benefit towards the objective of the study. The IR analysis indicates shift in peaks for Efavirenz in its inclusion complexes or precipitates but retention of characteristic peaks for the drug indicates no significant incompatibility between the drug and the carriers used. The DSC analysis shows decrease in peak intensity and homogeneous distribution of drug in the polymer carrier. The XRD analysis shows no diffraction pattern for the drug carrier precipitate indicating conversion of Efavirenz to amorphous or partially amorphous form.

This enhancement of dissolution of Efavirenz from drug carrier systems can be ascribed to several factors. Lack of crystallinity, i.e. amorphization, increased wettability and dispersibility and particle size reduction are considered to be important factors for dissolution rate enhancement. As indicative from dissolution data of physical mixtures, improvement could be attributed to higher wettability and dispersibility. Physical mixing of drug with a hydrophilic carrier results in greater wetting and increases surface available for dissolution by reducing interfacial tension between hydrophobic drug and the dissolution media. Furthermore, the drug micro crystals are embedded in the water-soluble matrix. Thus the hydrophilic carrier which presents the ability of rapidly dissolving in the dissolution medium causes rapid wetting of Efavirenz, leading to an improvement in its dissolution rate. Moreover, hydrophilic carrier encircling the hydrophobic drug decreases aggregation and agglomeration of drug particles, allowing a faster dissolution process.

CONCLUSION

Nano-sizing by liquid anti solvent precipitation technique is a classical approach to enhance solubility and dissolution rate of poorly water-soluble drugs. In the present work, the micro or nano-crystals were found to have improved solubility and dissolution rate of Efavirenz. The solubilization effect of β-CD or HP β-CD may be due to the structure of the drug with the phenyl ring that fit into the cyclodextrin cavity and the complex association, strengthened by hydrogen bonding between the amino group of the drug and the OH-groups of CDs. In addition, reduction of particle aggregation of the drug, formation of microcrystalline or amorphous drug, increased wettability

and dispersibility, and altered surface properties of the drug particles also contribute to the enhanced solubility and dissolution rates [12-14].

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