Original Research Article

Dissolution enhancement of efavirenz by solid dispersion and PEGylation techniques

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Abstract

Background: Efavirenz is the preferred nonnucleotide reverse transcriptase inhibitor for first-line antiretroviral treatment in many countries. It is orally active and is specific for human immunodeficiency virus type 1. Its effectiveness can be attributed to its long half-life, which is 52–76 h after multiple doses. The drug is having poor water solubility. The formulation of poorly soluble drug for oral delivery will be one of the biggest challenges for formulation scientists in the research field. Among the available approaches, the solid dispersion technique has often proved to be the most commonly used method in improving dissolution and bioavailability of the drugs because of its simplicity and economy in preparation and evaluation. **Materials and Methods:** Solid dispersions were prepared by solvent evaporation and physical mixture methods by using polyethylene glycol as the hydrophilic carrier and PEGylated product was also prepared. The prepared products were evaluated for various parameters, such as polymer interaction, saturation solubility study, and drug release studies. The drug release data were analyzed by fitting it into various kinetic models. **Results:** There is an improvement in the dissolution from 16% to 70% with solid dispersion technology. Higuchi model was found to be the best fit model. **Conclusion:** Solid dispersion is the simple, efficient, and economic method to improve the dissolution of the poorly water-soluble drugs.

Key words: BCS class II drugs, polyethylene glycol, solvent evaporation method

INTRODUCTION

An estimated 36 million people are infected with human immunodeficiency type-1 (HIV-1) worldwide.[1] Introduction of highly active antiretroviral therapy has brought with it significant reduction in mortality and opportunistic events, even in patients with very advanced stage of HIV infection.[2] Efavirenz, a nonnucleoside reverse transcriptase inhibitor is commonly used in therapeutic protocols to treat HIV patients.[3] It is a crystalline lipophilic solid with an aqueous solubility of $9.0\,\mu g/mL$ and with a low intrinsic dissolution rate (IDR) of $0.037\,mg/cm^2/min$.[4] The

drugs with less than $0.1\,mg/cm^2/min$ of IDR have dissolution as a rate-limiting step in absorption. This suggests the importance of dissolution improvement for efavirenz. Moreover, most of these new chemical entities despite their high permeability, are only absorbed in the upper small intestine. Consequently, if these drugs are not completely released in gastro intestinal tract area, they have low bioavailability.[5]

Chemical structure of efavirenz [Figure 1]
The US has defined a Biopharmaceutical Classification System (BCS) in which drugs are divided into 4 classes based on their solubility and permeability as shown below [Table 1].[5]

According to BCS, drugs with low aqueous solubility and high membrane permeability, such as efavirenz, are categorized as Class II drugs.[6-8] The dissolution of various drugs can be improved

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Table 1: Biopharmaceutical classification system

BCS Class I	BCS Class II
Highly soluble	Less soluble
Highly permeable	Highly permeable
BCS Class III	**BCS Class IV**
Highly soluble	Less soluble
Less permeable	Less permeable
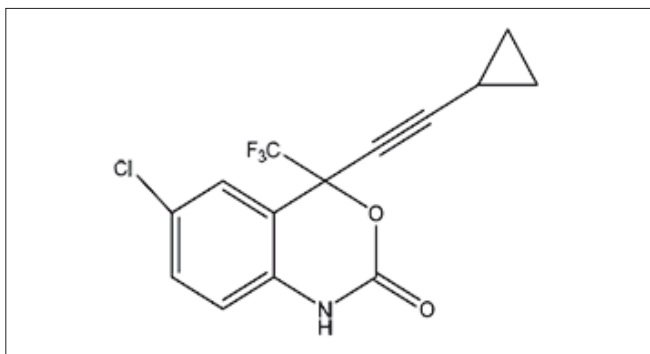
Figure 1: Chemical structure of efavirenz

by preparing the solid dispersions using suitable hydrophilic carriers. Solid dispersion technologies are particularly promising for improving the oral absorption and bioavailability of BCS Class II drugs. Solid dispersions are more efficient than these particle size reduction techniques, because the latter have a particle size reduction limit around 2–5 mm, which frequently is not enough to improve considerably the drug solubility or drug release in the small intestine and, consequently, to improve the bioavailability.[9] Drug solubilization from solid dispersion systems is mainly due to particle size reduction, increased surface area, reduction in crystallinity, and increased wettability by the surrounding hydrophilic carriers, which improve the dissolution rate.[10]

The PEGylation refers to the conjugation of drug moiety to polyethylene glycol (PEG) through covalent or noncovalent interaction and this increases the solubility of the drug.[11] The need and objective of our study is to enhance the dissolution rate of Efavirenz by using PEG.[12-14]

MATERIALS AND METHODS

Efavirenz was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India. PEG 6000 was obtained from SD Fine Chemicals Limited, Mumbai, India. All other reagents used were of analytical grade and UV Spectrophotometer (Elico SL159, Ahmedabad, India), USP Dissolution apparatus (Electrolab TDT 08L, New Mumbai, India), and Gyratory Shaker (DC Motors, Gujarat, India) were used.

Preparation of co-ground mixtures
The physical mixtures were prepared by triturating drug and PEG 6000 in a mortar with a pestle for 30 min.

Preparation of solid dispersions
The solid dispersions were prepared by using solvent evaporation method. These were prepared with drug:carrier (PEG 6000) ratios of 1:1 and 1:2 w/w. In solvent evaporation the drug and the carrier were added to a common solvent (ethanol) and were homogenized for 5 min. The solvent was removed using rota evaporator and dried at room temperature. The samples were pulverized using a mortar and pestle and passed through sieve no. 22 of 150 μm.[15]

PEGylation
The drug–PEG conjugates in 1:1 and 1:2 w/w ratios were prepared by dissolving efavirenz and PEG 6000 separately in possible minimum volumes of acetone. The acetonic solution of the drug was poured into the acetonic solution of PEG 6000 while stirring. The reaction mixture was incubated overnight at room temperature and acetone was evaporated by using rota evaporator to yield the PEGylated compound.[11,16]

Compatibility studies
Fourier transform infrared (FTIR) spectra were obtained using the FTIR spectrometer (Shimadzu, Japan) by the conventional KBr pellet method. The samples were ground gently with anhydrous KBr and compressed to form a pellet. The scanning range was 400–4000 cm^{-1} and the resolution was 4 cm^{-1}.

Differential scanning calorimetry studies
The samples of pure drug and products of 1:1 ratio formed by solid dispersion technique and PEGylation were studied for comparison of crystallinity with differential scanning calorimetry (DSC).

Phase solubility studies
The solubility of the drug was measured in the presence of 1%, 2%, and 3% up to 10% w/v PEG in distilled water. An excess amount of the drug was then added to approximately 10 mL of either distilled water or the carrier solutions in glass tubes. The tubes were kept under vibration for 24 h by using Gyratory Shaker (DC Motors, Gujarat, India). After reaching the equilibrium status, the saturated solutions were filtered through a 0.45-μm membrane filter, diluted with water, and then assayed spectrophotometrically.[17]

The values of apparent stability constant, K_s, between each drug–carrier combination were computed from the phase solubility profiles, as described below:

$$K_a = \frac{\text{slope}}{\text{intercept } (1 - \text{slope})}$$

The Gibbs free energy of transfer (ΔG) of efavirenz from pure water to aqueous solutions of solubilizing agents was calculated using the following equation[18]:

$$\Delta G = -RT \ln (S_s/S_w)$$

Where S_s/S_w is the ratio of molar solubility of the drug in aqueous solution of PEG to that of the pure water, R the gas constant (8.3143 J/K/mol), and T the absolute temperature (K).

In vitro dissolution studies
In vitro dissolution testing employed the United States Pharmacopeia Apparatus II (Electrolab TDD-08L, New Mumbai, India) at 50 rpm with 900 mL of water with 0.1% Tween 80 at 37°C ± 0.5°C. A powder sample equivalent to 50

mg of the drug filled into capsules was tested. The sample of the dissolution media was removed at predetermined time intervals and was simultaneously analyzed spectrophotometrically at a λ_{max} of 247 nm.

Kinetic analysis of dissolution data

To study the mechanism of drug release from the formulations, the release data were fitted to the following equations.

Zero order kinetics: Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly follows.

$$Q_1 = Q_0 + K_0 t$$

Where, Q_1 is the amount of drug dissolved in time t, Q_0 the initial amount of drug in the solution, and K_0 is the zero order release constant.

First order kinetics: This model has been used to describe absorption and/or elimination of some drugs.

$$\ln Q_t = \ln Q_0 K_1 t$$

Where, K_1 is the first order release constant, Q_0 the initial amount of drug in the solution, and Q_1 is the amount of drug dissolved in time t.

Higuchi model: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms.

$$Q = [t\, D\, C_s\, (2C - C_s)]^{1/2}$$

Where, Q is the amount of drug release in time t, C the initial drug concentration, C_s the drug solubility in the matrix, and D is the diffusion constant of the drug molecule in that liquid.

Korsmeyer–Peppas: This model is generally used to analyze the release of pharmaceutical polymeric dosage from, when more than one type of release phenomena could be involved.

$$Q_t/Q_\infty = a\, t^n$$

Where, a is the constant incorporating structural and geometric characteristics of the drug dosage form, n the release exponent (indicative of the drug release mechanism), and Q_t/Q_∞ is the fractional release of the drug.

The criterion for selecting the most appropriate model was based on a goodness-of-fit test.[19]

RESULTS AND DISCUSSION

Compatibility studies
From the FTIR studies shown in Figure 2, it is very clear that there are no interactions between drug and PEG. All the peaks responsible for the active functional groups were even present in the FTIR spectra of the drug along with PEG. According to the work of Afrouz *et al.*, 2009,[16] FTIR peak near 1650 cm⁻¹ indicates the formation of PEG bond with the drug. In FTIR spectrum of the drug with PEG, there is a peak near 1650 cm⁻¹, indicating the PEGylation.

Differential scanning calorimetry studies
DSC curves in Figure 3 revealed that both PEG and the drug
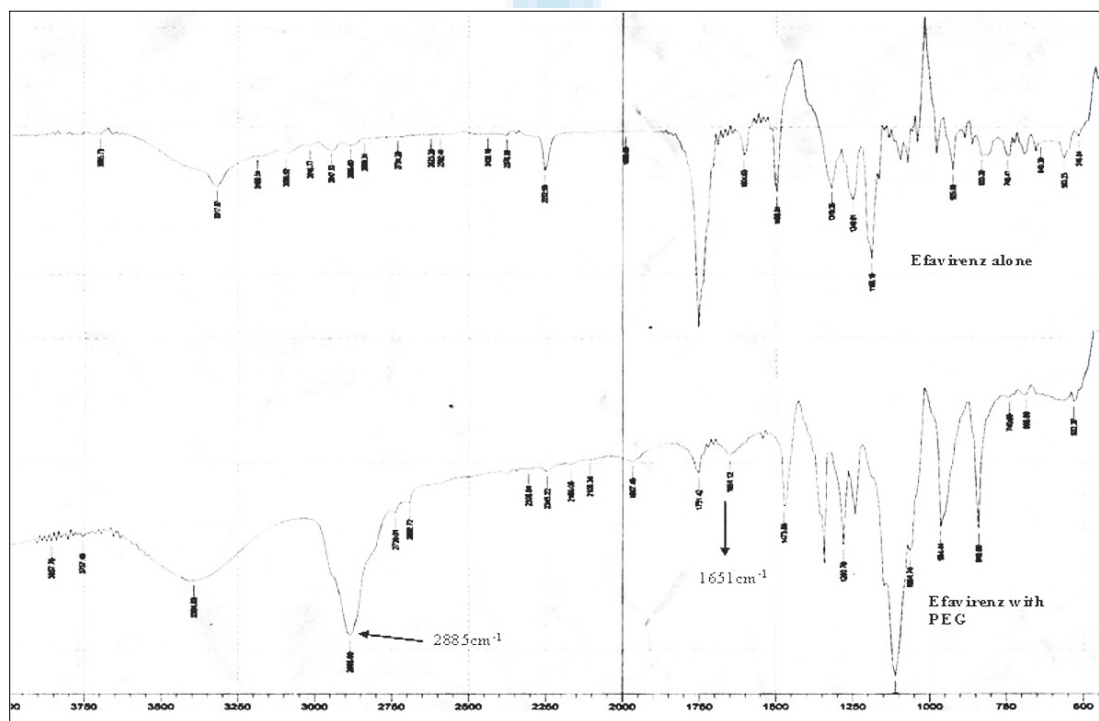
Figure 2: Fourier transform infrared of pure drug and fourier transform infrared of drug along with PEG

Table 2: Calibration curve

Concentration (µg/mL)	Absorbance
1	0.02
2	0.077
3	0.074
4	0.128
5	0.126
15	0.321
20	0.412
25	0.567
30	0.606
R^2 value	0.9907
Slope	0.0201
Intercept	0.02385

$Y = 0.0201X + 0.02385$

Table 3: Phase solubility data

Concentration of PEG (mg/mL)	Amount of drug dissolved in mg at room temperature	ΔG (J/mol)	K_s (M^{-1})
0	0.000	0	643
1	2.303	−744	
2	2.596	−1041	
3	3.104	−1484	
4	3.945	−2078	
5	4.409	−2353	
6	4.887	−2608	
7	5.419	−2864	
8	5.986	−3111	
9	6.364	−3263	
10	6.818	−3434	

PEG – Poly ethylene glycol

Figure 3: Differential scanning calorimetry curves of pure drug, solid dispersion, and PEGylated product of drug at 1:1 ratio with PEG 6000
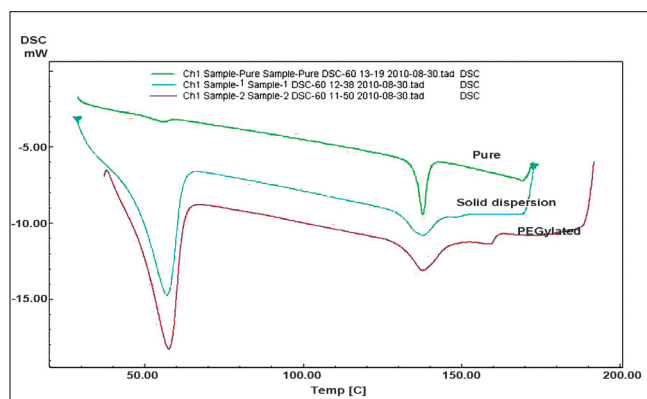

Figure 4: Standard calibration curve of pure drug

exhibited an endothermic peak with the onset temperature around 57.5°C and 137.27°C, respectively. Efavirenz has original peak onset and offset as 135.27°C–139.79°C. In PEGylated product and solid dispersion, there was broadening of peak with onset and offset between 129.7°C–142.5°C and 130.8°C–144.57°C, respectively. The heat of fusion was 41.64, 46.41, and 67.79 J/g for pure, PEGylated compound, and solid dispersion, respectively. From the thermograms of solid dispersion and PEGylated product, the reduction of peak area for drug can be observed. This indicates the amorphous nature of the drug in a molten carrier.
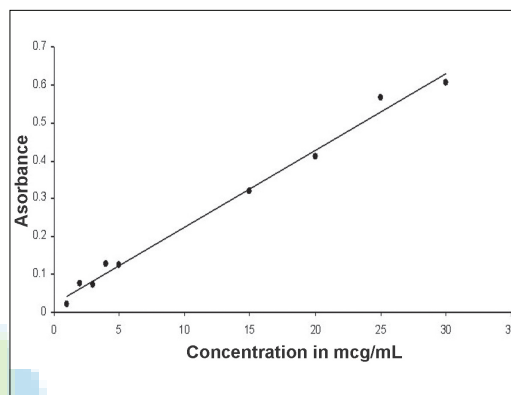
Calibration curve

Calibration curve was prepared in phosphate buffered saline with 0.1% Tween 80. The coefficient of correlation [R^2] value was found to be 0.9907, and because it was above 0.99, the calibration curve values are acceptable. The slope was with a value of 0.0201. The intercept of the curve was positive and its value was 0.02385. It is shown in Table 2 and Figure 4.

Phase solubility studies

From the phase solubility studies in Table 3 and Figure 5, it was found that with the increased concentrations of the PEG, the solubility of efavirenz had increased. This indicates that PEG can be used as a solubility-aiding agent for the drug efavirenz. The type of curve formed is A_L type, which indicates a positive linear effect on the solubility of the drug with increased concentration of PEG.

The Gibbs free energy values were calculated and were given in Table 3. These values were negative and were decreased with increase in the concentration of PEG. The negative nature of the Gibbs free energy changes is an indicative of the spontaneity of the process. The apparent stability constant was found to be 643 M^{-1}. The thermodynamic Gibbs free energy values also confirm the positive effect of PEG on dissolution of the drug.

In vitro drug release studies

After observing the percentage of drug release shown in Table 4 and Figure 6 up to 100 min of all the formulations, the order of enhanced dissolution is as follows:

Pure (16%) < physical mixture 1—1:1 ratio of drug and PEG 6000 (30%) < physical mixture 2—1:2 ratio of drug and PEG 6000 (38%) < PEGylated compound 1—1:1 ratio of drug and PEG 6000 (54%) < PEGylated compound 2—1:2 ratio of drug and PEG 6000 (56%) < solid dispersion 1—1:1 ratio of drug

## Table 4: Cumulative % drug release data of all the formulations

Time (min)	Cumulative % drug release												
	Pure	±SD	PM1 (1:1)	±SD	PM2 (1:2)	±SD	PEG1 (1:1)	±SD	PEG 2 (1:2)	±SD	SD 1 (1:1)	±SD	SD2 (1:2)	±SD
5	3	0.52	1.5	1	9	2.29	12	3.88	17	0.97	0.9	0.62	0.04	0.01
10	3.2	0.16	1.9	1.3	10	3.09	23	1.71	22	0.24	2.1	0.86	0.2	0.47
15	6.9	0.17	2.9	0.8	20	1.19	30	2.58	32	3.29	5.2	1.89	3.6	0.21
20	8.5	0.21	5.1	1.2	22	1.27	32	1.46	34	0.01	11.9	1.73	12.5	1.73
25	9.6	0.26	9.2	1.2	23	1.19	33	0.83	39	2.08	13.5	0.77	19.5	1.06
30	10.9	0.22	13	1.2	26.8	0.43	40	1.39	42	0.05	26.4	0.67	33.5	2.47
45	15.2	0.83	19	1.1	31.5	1.24	42	0.14	48	2.09	42.1	1.17	46.5	1.77
60	15.3	0.19	23	1.9	34.5	1.76	49	1.98	50	1.55	53.1	2.37	56.9	1.34
80	17	0.01	27	1.2	36.7	1.28	51	1.63	54	1.97	64.1	1.47	61.5	1.06
100	17.1	0.52	32	1.1	39.8	0.82	54.9	0.48	57	0.61	72.1	1.41	72.9	1.2

PM, physical mixtures; PEG, PEGylated compound; SD, solid dispersions; SD, standard deviation

Table 5: Kinetic model fitting for the drug release data

Model	PM 1	PM 2	PEG 1	PEG 2	SD 1	SD 2
Zero order	0.9192	0.9610	0.9511	0.9153	0.9685	0.9696
First order	0.8435	0.9115	0.8971	0.8397	0.7381	0.7192
Higuchi	0.9712	0.9908	0.9874	0.9689	0.9777	0.9831
Korsmeyer–Peppas	0.9798	0.9924	0.9917	0.9799	0.9362	0.9262
n Value	0.5013	0.4024	0.3938	0.4232	2.5388	2.5134

PM, physical mixtures; PEG, PEGylated compound; SD, solid dispersions
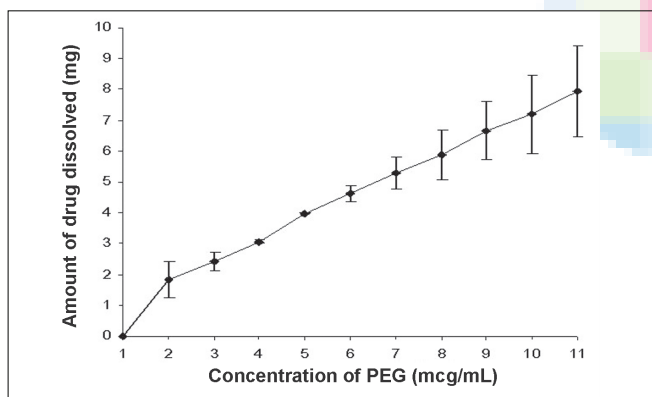
Figure 5: Phase solubility curve for efavirenz with PEG
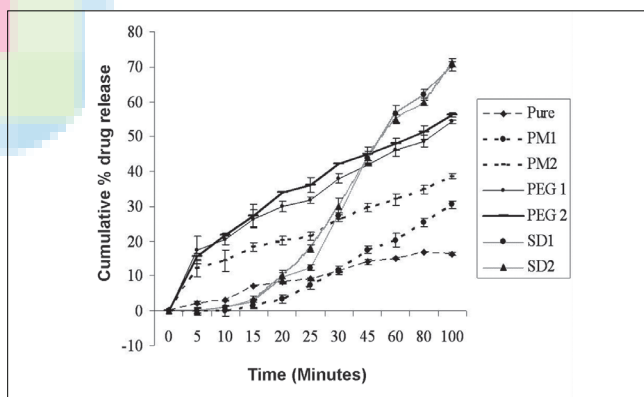
Figure 6: *In vitro* drug release

and PEG 6000 by solvent evaporation (70%) < solid dispersion 2—1:2 ratio of drug and PEG 6000 by solvent evaporation (71%).

This indicates that a simple physical mixture is not sufficient for the reduction in the hydrophobic surface of the drug. Even in the PEGylation process, the formation of PEG chains on the surface of hydrophobic surface of the drug could not achieve the drug release as high as it is in solid dispersions. The drug release was found to be increased tremendously in solid dispersions up to 70% from 16%. In solid dispersions, there is a chance for the formation of hydrophilic matrix of PEG in which the drug gets entrapped in the amorphous state. As the hydrophilic matrix depletes, the drug in the amorphous state will be available for

dissolution. This can be contributed as a reason for the highest increase in dissolution by solid dispersion technique.

When the data were fit into various kinetic models, it was found that the matrix model is the best fit model for all [Table 5]. The n value for the model is less than 0.5 in all the formulations except solid dispersions, indicating the mechanism of release as the diffusion with Fick's law. In solid dispersions, the n value is above 0.5 (near to 2.5), indicating the mechanism of release as diffusion, which follows non-Fickian laws. The formation of the hydrophilic matrix of the carrier might be the reason for the diffusion of drug from the matrix that follows the non-Fickian kinetics.

CONCLUSIONS

The present work clearly shows that the addition of PEG 6000 to drug improves its dissolution rate. The mechanism involved may be the solubilization and improved wetting of the drug in the PEG-rich microenvironment formed at the surface of drug crystals after dissolution of the polymer. The crystallinity of the drug was reduced in both solid dispersion and PEGylated compound. Formulation of solid dispersions and PEGylated compounds improved dissolution rate compared with physical mixtures. The results indicate that the dissolution rate of the water-insoluble drug efavirenz can be enhanced significantly by the simple solid dispersions using the hydrophilic carriers, such as PEG than PEGylation technology.

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