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Enhancement of solubility and dissolution rate of poorly water-soluble Etravirine by solid dispersion technique for antiretroviral therapy

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ABSTRACT

Solid dispersions (SDs) technique represents a promising approach to enhance the solubility, dissolution rate and bioavailability of poorly water-soluble drugs. Etravirine is a new non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immune deficiency (HIV) virus 1 and it belongs to class IV in Biopharmaceutics classifications system (BCS). The major problem with this drug is its poor solubility in biological fluids, which results in poor bioavailability after oral administration. In the present study, five formulations of etravirine solid dispersion were formulated by solvent evaporation technique using Hydroxypropyl methylcellulose (HPMC), Polyethylene glycol (PEG 6000) as hydrophilic carriers with the ratio of 1:1 and 1:2. The dissolution result shown that there was a significant increase in the solubility of etravirine from all formulations. It was observed that formulation (SD2) comprising Etravirine: HPMC (1:2) ratio has shown enhanced solubility and faster dissolution rate. This may due to the conversion of crystalline to an amorphous form of etravirine in solid dispersion consists of a hydrophilic carrier and also increase in wettability. Hence the study was concluded that etravirine SDs could be beneficial for the treatment of HIV/AIDS with enhanced dissolution rate and bioavailability.

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INTRODUCTION

Poor solubility or dissolution rate behaviour of existing and newly developing drugs remains a challenge in the development of efficient drug delivery system which leads to a reduction in oral bioavailability. This is due to a reduction in both the rate and extent of drug absorption. It has been reported that approximately 40% of newly discovered drug moiety has poor aqueous solubility. Unfortunately, most of these drugs are unrepressed in prior stages of progress because of the solubility difficulties. Hence it is essential to control the solubility problems of these nonsoluble drugs with improved pharmacological effects. (Lipinski, CA. 2000, Lipinski CA, *et al.,* 2001)

Various technologies have been developed to enhance the solubility of poorly soluble drugs such as solid dispersion, hydrography, nanocrystals, lipid formulations, solubilization using a surfactant, reduction of particle size and cyclodextrin complexes (Yanbin Huanga *et al.,* 2014). Among these various approaches, solid dispersion is the most efficient one which involves dispersion of one or more active ingredients in an inert carrier or matrix in the solid state. It has been reported that the possible mechanisms for the enhancement of the dissolution rate of solid dispersions are molecularly dispersed drugs in polymeric carriers may achieve the highest levels of particle size reduction and surface area enhancement, which result in enhancement of dissolution rates. Furthermore, no energy in

Table 1: Composition of Etravirine solid dispersions by a Solvent evaporation method

S.No	Formula Code	Etravirine (mg)	Polymers (mg)		SLS (mg)
			HPMC	PEG 6000	
1.	SD_1	200	200	-	200
2.	SD_2	200	400	-	200
3.	SD_3	200	-	200	200
4.	SD_4	200	-	400	200
5.	SD_5	200	400	400	200

Table 2: Preliminary solubility studies of etravirine and polymers

S.NO	Formula code	Drug : HPMC:PEG 6000 (200:200:200mg)	Product description	Formulation nature	Method of preparation
1.	Pure drug	1: 0	--	White crystals	--
2.	PM1	1:1:0	Physical Mixture	Off-white sticky particle	--
3.	PM2	1:2:0	Physical Mixture	Off-white sticky particle	--
4.	PM3	1:0:1	Physical Mixture	Off-white sticky particle	--
5.	PM4	1:0:2	Physical Mixture	Off-white sticky particle	
6.	PM5	1:2:2	Physical Mixture	Off-white sticky particle	
5.	SD1	1:1:0	Solid dispersion	Free flow powder	
6.	SD2	1:2:0	Solid dispersion	Free flow powder	
7.	SD3	1:0:1	Solid dispersion	Free flow powder	Solvent evaporation method
8.	SD4	1:0:2	Solid dispersion	Free flow powder	
9.	SD5	1:2:2	Solid dispersion	Free flow powder	

needed to break up the crystal lattice of a drug during the dissolution process, and drug solubility and wettability may be increased by surrounding hydrophilic carriers (Manvi, P *et al.,* 2011).

Acquired immune deficiency syndrome (AIDS) is a degenerative disease of the immune system caused by the human immunodeficiency virus (HIV), a lentivirus belonging to the family of the Retroviridae (Araujo, A *et al.,* 2003). There were approximately 34 million people living with HIV in 2010. In 2010, around 6.6 million people living with HIV were receiving antiretroviral therapy in low- and middle-income countries, but over 7 million others are waiting for access to treatment (WHO, 2011). Some 3.4 million children are living with HIV - many of whom lack HIV treatment. Of the 4.8 million people living with HIV in Asia, nearly half (49%) are in India (WHO, 2011).

Understanding the mode of HIV infection and the availability of different potent drugs has mandated highly active anti-retroviral therapy (Tripathi, K. 2013). At present, there are over 20 different anti-retroviral drugs approved under the general classes of nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), fusion inhibitors (Vyas T *et al.,* 2008). Some other classes of drugs like Zinc finger inhibitors, Antisense drugs, CD4 receptor antagonists, CCR5 antagonist. There are several anti-retroviral available in the market today which includes zidovudine, zalcitabine, didanosine, lamivudine, stavudine, and abacavir, etravirine and many others (Gupta, S *et al.,* 2007, Joly, V, *et al.,* 2000). Among these drugs, etravirine plays a major role to reduce the viral infections.

Etravirine is a novel second-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), chemically 4-[6-Amino-5- bromo-2-[(4-cyanophenyl) amino] pyrimidin-4-yl] oxy- 3, 5-dimethyl benzonitrile which acts by molecularly blocking the viral reverse transcriptase enzyme. It achieves this by preventing the enzyme from converting its genetic material (RNA) into proviral DNA and thus preventing incorporation of the viral genome into

Table 3: Preliminary solubility studies of etravirine and various hydrophilic polymers

S.NO	Sample	Solubility (mg/ml)
1.	Etravirine	0.06±0.02
2.	Etravirine: Xanthum Gum	0.13±0.18
3.	Etravirine: Chitosan	0.09±0.03
4.	Etravirine: EC	0.17±0.05
5.	Etravirine: HPMC	0.24±0.01
6.	Etravirine: PEG 6000	0.20±0.02
7.	Etravirine : HPMC: PEG 6000 (1:2:2) Ratio	0.21±1.12

Mean±S.D, n=3

the human host cell (Andries, K. *et al.,* 2004, Vingerhoets, J., 2005).

Etravirine 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.** It belongs to BCS class-IV. It is not soluble in water and practically insoluble across the pH range of 1 - 6.8. The major concern with the Etravirine is its low solubility, which results in low bioavailability after oral intake. (TN, Kakuda. 2008) The solubility of such drugs can be improved by incorporating the drug in a matrix of the hydrophilic carrier(s) obtaining a product called a solid dispersion.

Considering the above factors, the present study proposed to formulate and quantify a novel solid dispersion of etravirine. Here the poorly soluble etravirine can be solid dispersed that enhances dissolution and also absorption rate. As yet no reports are available in the progress of etravirine solid dispersion with hydrophilic polymers. Thus, an effort has been created for its solubility improvement.

MATERIALS AND METHODS

Etravirine gifted from Par pharmaceutical, Chennai, India. Hydroxypropyl methylcellulose (HPMC) was procured from Loba Chemie Pvt, Ltd, Mumbai, Polyethylene glycol 6000 (PEG 6000) was procured from Moly Chem, Mumbai. Concentrated hydrochloric acid was purchased from Ranbaxy Fine Chemicals, Vijayawada (India). Anhydrous sodium sulphate was procured from Samir Tech Chem, Makarpura (Vadodara). Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from S.D. Fine Chemicals, Bangalore (India). Methanol and water were procured from Merck, Bangalore (India). All the chemicals used were of analytical grade. All solutions were prepared using double distilled water.

Preparation of a physical mixture of solid dispersions

Physical mixtures were prepared by mixing the appropriate amount of Etravirine and Hydroxypropyl methylcellulose (HPMC) and Ethyl Cellulose in mortar and pestle separately and passed through sieve # 60. The obtained material was kept in a desiccator to carry out further examination (Ramesh, K *et al.,* 2015).

Preliminary solubility studies of Etravirine

Solubility study of etravirine was performed by the given method. Initially, one part of Etravirine was added to 25ml of an aqueous solution of water-soluble natural and semi-synthetic carriers like Xanthum gum, chitosan, dextran (Ethylcellulose (EC), HPMC, PEG 6000 in 1:1 ratio with an equal proportion of Sodium lauryl sulphate (SLS) and were taken in screw-capped bottles. 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 in UV/Visible spectrophotometer at 235 nm (Ramesh, K *et al.,* 2015).

Preparation of solid dispersions of Etravirine by a solvent evaporation method

Etravirine solid dispersions of five formulations were prepared by using carriers shown in Table 1 like hydroxyl propyl methyl cellulose (HPMC), PEG 6000 at 1:1 ratio etc., with a surfactant, i.e., Sodium lauryl sulphate (SLS) in proportions viz. 1:1:1, 1:2:1 (Drug: Carrier: Surfactant). The drug and carrier along with SLS were dissolved in Methanol and triturated in a dry mortar until the solvent is evaporated and a clear film of drug and carrier was obtained. Then the dispersion was subjected to Methanol solvent evaporation by placing in a vacuum dryer at 50°C chamber for 30 min period. The resultant solid dispersion was scraped out with a spatula. Solid dispersions were pulverized in a mortar and pestle and passed through a 420 µm (ASTM #40 mesh) mesh before packing in an airtight container (Ramesh, K *et al.,* 2015)

Fourier Transform Infrared Spectroscopy Studies (FTIR)

This study was performed to examine the interaction between drug and polymers in the formulations. FTIR spectrum of pure drug, physical mixtures and solid dispersions were recorded by using FT-IR 8400S (SHIMADZU, Kyoto, Japan) with the scanning range 4000 to 400cm^{-1} (Ramesh, K *et al.,* 2015).

% Practical Yield

Percentage of practical yield was calculated to know about percent yield or efficiency of any method, thus its help in the selection of the appropriate method of production. SDs were collected and weighed to determine effective yield (PY) from the following equation (Ramesh, K *et al.,* 2015).

$$\%\,\text{Practical yield} = \frac{\text{Practicalweight (soliddispersion)}}{Th9{:}r9t{:}cal\ A9{:}ght\ (p{:}lym9r + drug + surJactant)} \times 100$$

Drug content analysis

The stable dispersion of equivalent to 10 mg of etravirine was measured precisely and transported to 100 ml volumetric flask and dissolved in 0.01M HCl. The volume was made up to the mark with 0.01M HCl. The absorption maxima (λmax) of the above solution were measured at 235 nm after making suitable dilution using an appropriate blank solution. The drug content of etravirine was calculated using a calibration curve (Ramesh, K *et al.,* 2015).

In-vitro drug release studies

The *in-vitro* drug release profile of an entrapped drug postulates the function of the delivery system. *The in-vitro* etravirine release profile for all formulations as well as a pure drug was performed using USP XXII type 2 dissolution apparatus (TDP-06P, Electro lab, Mumbai, India). Preparation equivalent to 100 mg of etravirine was taken in 900 ml of 0.01M HCl at 37± 0.5ºC and stirred at 50 rpm. Aliquot of 5ml was withdrawn at time intervals of 5, 10, 15, 20, 30, 45, 60 and 90 min. The withdrawn volume was replaced with the same volume of dissolution medium in order to keep the total volume constant. The absorbance of the samples was measured using spectrophotometrically at λmax 235nm after suitable dilution if necessary, using an appropriate blank (Ramesh, K *et al.,* 2015).

Statistical analysis

The data were analyzed by one way ANOVA followed by Tukey's multiple comparison tests with the help of Graph Pad Instat software, version 6.01. All the data were presented as a mean value with its standard deviation (mean±S.D). P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Preparation of solid dispersion

In the present study etravirine, solid dispersions were formulated using various hydrophilic polymers. The description, nature of physical mixture and formulations were given in Table. 2

Preliminary solubility studies of etravirine and various hydrophilic polymers

Preliminary solubility studies of Etravirine was performed with various natural and semi-synthetic hydrophilic carriers such as xanthum gum, chitosan, EC, HPMC, PVP6000 to select the appropriate water-soluble carriers for the preparation of solid dispersion in which etravirine solubility was found to be 0.06±0.02 mg/ml, and the results were given in Table. 3. From this study, it was observed that etravirine: HPMC in the ratio of 1:2 shows the highest solubility compared to other physical mixtures. The turbid solution was observed in natural polymers such as xanthum gum and chitosan. The solubility of etravirine with EC was less (0.17±0.05 mg/ml) when compared with other polymers, and it was given in Fig 1. Based on the observed results we have selected HPMC and PEG 6000 and its combination for our further studies.

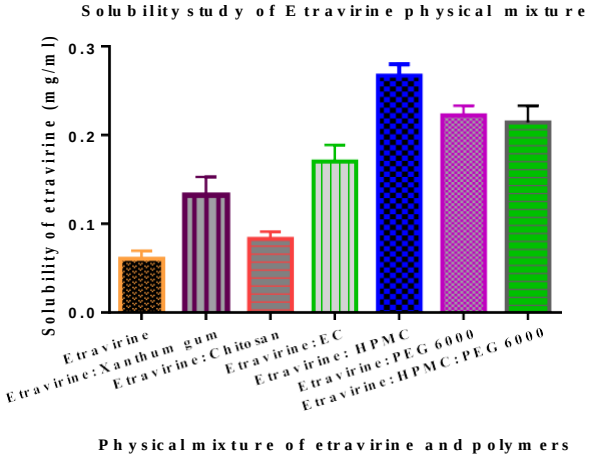

Figure 1: Preliminary solubility studies of etravirine and polymers in 0.01M HCL

Table 4: Solubility studies of Etravirine solid dispersions

S.NO	Formula Code	Solubility (mg/ml)
1.	Etravirine	0.06±0.021
2.	SD1	0.50±0.043
3.	SD2	0.78±0.018
4.	SD3	0.34±0.016
5.	SD4	0.58±0.034
6.	SD5	0.73±0.010

Mean±S.D, n=3

Preparation of Etravirine solid dispersions

Solid dispersions of Etravirine were prepared by a solvent evaporation method using HPMC AND PEG 6000 as polymers. In the present investigation, 12 formulations were prepared and their complete composition was shown in Table 1. The prepared solid dispersions were found to be fine and free-flowing powders.

Solubility studies of Etravirine solid dispersions

Various formulations of solid dispersions with different ratios of polymers were prepared by a

solvent evaporation method with their respective carrier along with a surfactant. Solubility studies on prepared solid dispersions were performed to know the correlation with the results observed from preliminary solubility studies. It was found that formulation SD2 (Drug: HPMC) in the ratio of 1:2 shown highest solubility, i.e. 0.78±0.018 compared to other formulation (Table.4). It was given in Fig.2.

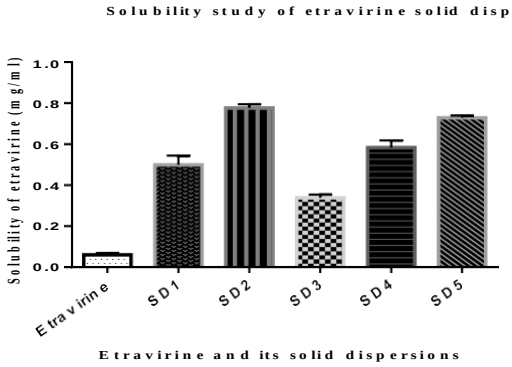

Figure 2: Solubility studies of etravirine solid dispersions

Fourier Transform Infrared Spectroscopy Studies (FTIR)

The FTIR results were given in Fig.3a, b, c, d, e. It was done to observe the interaction between drug and carrier used in the preparation of solid dispersion by potassium bromide disc method using Infrared Spectrophotometer. The obtained result showed that there was the absence of interaction between the polymer and pure drug used in solid dispersion formulations.

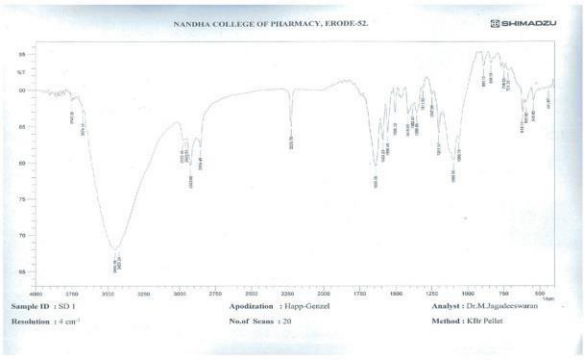

Figure 3a: FTIR of SD1

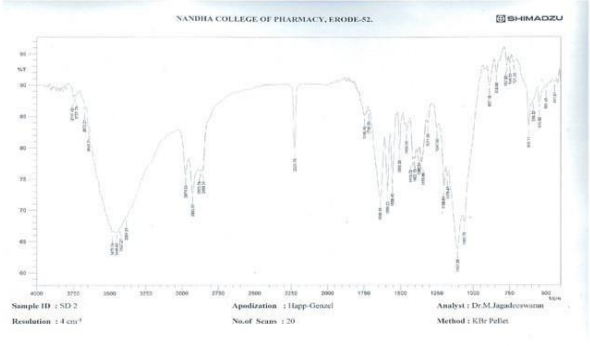

Figure 3b: FTIR of SD2

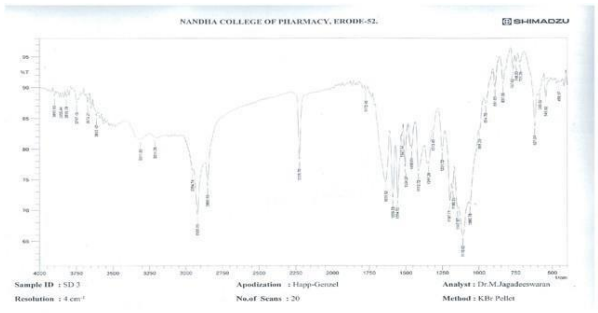

Figure 3c: FTIR of SD3

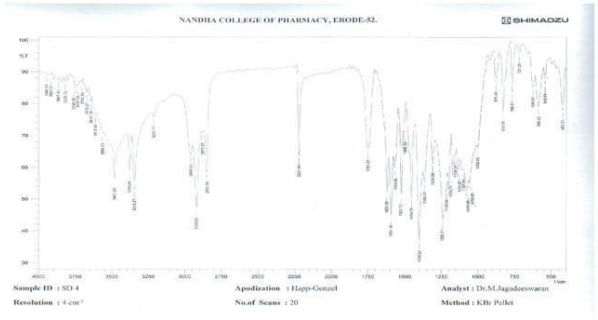

Figure 3d: FTIR of SD4

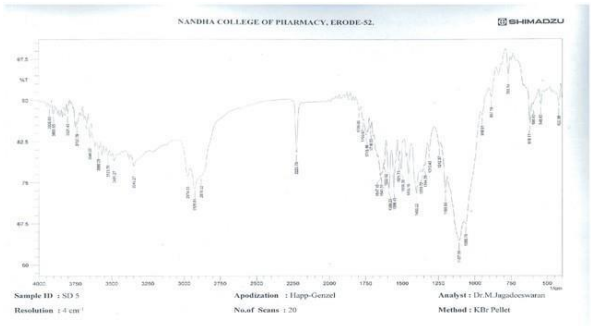

Figure 3e: FTIR of SD5

% Drug content and % Practical yield

The results of % drug content and % practical yield for all formulations are given in Table.4 and it was found to be 91.57±0.04 -98.68±0.07%. Maximum drug content was found in the formulation SD2. The % Practical yield of the prepared solid dispersions was found to be in the range of 87.41±0.02-97.05±1.20%. This may due to more wettability of HPMC with poorly soluble etravirine.

In- vitro % drug release characteristics

In- vitro % etravirine release characteristics were undergone to observe the dissolution enhancement character of polymers used in the formulation of the solid dispersion. The study was performed in 0.01N HCl. The % cumulative drug release data was given in Table.5. The obtained results reveal that the release of etravirine from solid dispersion is high when compared to pure (Fig.4). The *in- vitro* etravirine release data indicate that the formulation SD2 consists of HPMC as a polymer (1:2 ratio of Drug: HMPC) shows enhanced release

Table 5: %Drug content and % Practical Yield

S.No	Formulation Code	% Practical Yield	% Drug Content
1.	SD1	89.17±0.81	92.11±0.24
2.	SD2	97.05±1.20	98.68±0.07
3.	SD3	87.41±0.02	91.57±0.04
4.	SD4	92.17±0.74	93.38±2.02
5.	SD5	93.17±0.81	95.05±1.32

Mean±S.D, n=3

Table 6: In- vitro dissolution profiles of etravirine and solid dispersions (SD1-SD5)

Time (min)	Cumulative % drug release					
	Etravirine	SD1	SD2	SD3	SD4	SD5
5	12.3±1.2	24.3±0.4	38.5±0.5	21.2±0.03	26.1±0.01	30.5±1.9
10	18.8±0.01	39.3±2.5	59.1±0.04	28.5±0.1	38.2±0.5	36.4±1.03
20	27.8±0.6	58.5±0.4	65.0±0.01	41.3±0.4	57.8±0.3	58.3±1.1
30	30.9±0.5	69.8±0.03	78.5±0.2	58.1±0.01	64.9±1.8	69.2±1.01
45	32.8±2.3	75.8±0.8	87.0±1.5	63.2±2.5	72.1±0.6	83.1±1.5
60	38.4±0.3	82.7±0.1	95.0±2.1	79.5±0.9	81.5±0.01	85.4±0.01
90	45.2±0.9	89.5±1.6	99.1±0.01	86.5±0.7	91.6±0.5	93.8±1.8

Mean±S.D, n=3

characteristics when compared to other solid dispersions formulations in 0.01N HCl. The probable rationale for the gradual increase in solubility of etravirine in solid dispersions may be an enhancement of wettability of drug with hydrophilic polymer and transformation of the drug from crystalline to amorphous form.

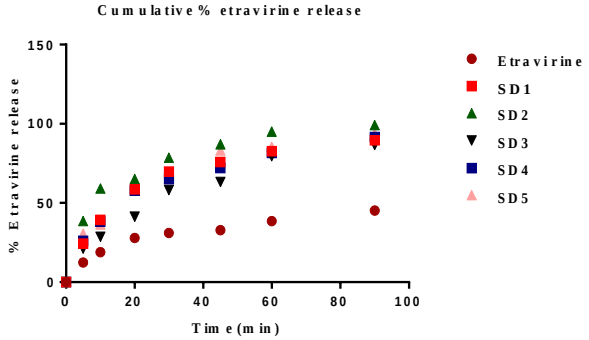

Figure 4: Cumulative % of etravirine release

CONCLUSION

Based on results observed, this study state that the solid dispersion of poorly soluble etravirine with HPMC as a carrier in the ratio of 1:2 has highest release rate and this composition would be favourable for the treatment of HIV/AIDS with enhanced release rate and also absorption. Extrapolation of the findings of the present study to animals may address the *in-vivo* bioavailability and *in-vitro* - *in-vivo* correlation of this approach.

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