Research Article

FORMULATION AND EVALUATION OF SOLID DISPERSION OF LOPINAVIR BY USING DIFFERENT TECHNIQUES

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ABSTRACT

Poor aqueous solubility is the major drawback for the various types of drugs and many approaches have been introduced for the enhancement of solubility of such drugs. Solid dispersion is one of the technique adopted for the formulation of such drugs. The main objective of the present investigation was to improve the Solubility and dissolution rate of Lopinavir (LVR), a poorly water-soluble drug by solid dispersion technique using a water-soluble carrier, Soluplus. Among the various solid dispersion techniques Solvent Kneading Method used to prepare the solid dispersion in various ratios such as 1:0.5, 1:1 and 1:2. The differential scanning Calorimetry and x-ray diffraction studies demonstrated that enhanced solubility and dissolution rate from solid dispersion due to the decrease in the crystallinity. Solid dispersions were characterized for drug content, solubility study, dissolution behavior, XRD study, DSC study and SEM analysis. Finally Solid dispersion was formulated into conventional dosage form such as Tablet.

Keywords: Lopinavir, Soluplus, Solubility, Dissolution, Solid Dispersion, Immediate Release Tablet.

INTRODUCTION

Newly discovered chemical molecules have high therapeutic activity but have low aqueous solubility which results in poor absorption and bioavailability. Many methods were reported for solubility and dissolution enhancement of poorly soluble drug such as micronization, complexation, particle size reduction, etc. However, all this methods have limitations like micronized powder having high energetic surface, which shows poor flow property and particles often agglomerated. Complexation with cyclodextrin shows low drug load and limitations for drug selection.¹ Solid dispersion technique is used in the pharmaceutical field to enhance the solubility and dissolution rate of poorly aqueous soluble drugs. It has been used widely to improve the oral absorption and bioavailability of BCS class II drugs. Solid dispersion is defined as ‘a dispersion of drug molecules in an inert carrier or matrix in the solid state.’² Low oral bioavailability of drug may be due to poor aqueous solubility, high first pass metabolism and efflux transport.³

Lopinavir is a potent protease inhibitor used for the treatment of HIV infections, and it has become a leading component in combined chemotherapy commonly referred as Highly Active Anti-Retroviral Therapy (HAART). Lopinavir shows poor bioavailability when administered orally. The major reason for poor bioavailability is poor drug solubility characteristics, followed by its first pass metabolism.⁴

MATERIALS AND METHODS

Lopinavir was obtained as a Gift sample (Microlabs Ltd. Bangalore), carrier Soluplus was obtained as a Gift sample (Eumedia Pharma Pvt. Ltd. Mumbai), Mannitol, Sodium starch glycolate, Magnesium Stearate, Lactose, Talc, Ethanol, Hydrochloric acid, Methanol were purchased from S.D Fine Chem Ltd., Mumbai and Himedia

Laboratories Pvt. Ltd. Purified water obtained from MilliQ Plus (Millipore) was used for all experiment. All the carriers used were of analytical grade.

Preparation of solid dispersion

In order to optimize drug to carrier ratio, physical mixture, solvent evaporation and solvent kneaded mixture of Lopinavir with Soluplus were prepared in different ratios.^{5,6,7} (Table 1)

Physical Mixture

In this method, the drug Lopinavir and polymer Soluplus were simply mixed using spatula and gently triturated to get uniformly mixed drug polymer mixture. The mixture was prepared in ratio of 1: 0.5, 1:1, and 1:2 drug polymer respectively. The resultant mixture was stored until further use.

Solvent Evaporation

Solvent evaporation method involves the incorporation of drug and the polymer in ethanol and then entire solvent was evaporated. The mixture was prepared in ratio of 1:0.5, 1:1, and 1:2 drug polymer respectively were added in Ethanol in a beaker and the solution was vigorously stirred on the magnetic stirrer until the entire ethanol was evaporated to obtain a clear solvent free film.

Solvent Kneading

The mixture was prepared in ratio of 1:0.5, 1:1, and 1:2 drug polymer respectively. In this method, the physical mixture of Lopinavir and Soluplus was kneaded using methanol to form a thick paste. The thick paste obtained was then dried.

Evaluation of solid dispersion

FTIR Study

Drug-excipients compatibility study was done to study the interaction between drug and polymers used in the preparation of solid dispersion. FTIR spectra of pure drug Lopinavir, Polymer Soluplus, physical mixture of drug and polymer and Solid dispersion of optimised batch of selected method were recorded by using a Spectrophotometer (Shimadzu, IRAffinity-1, Kyoto, Japan). The samples were dispersed in dry potassium bromide (KBr). FTIR spectra were run between 500 cm^{-1} to 4000 cm^{-1} .

Saturation Solubility Study for Lopinavir and Solid Dispersion

For solubility determination, excess amount of Lopinavir and Solid Dispersion was added in vials containing distilled water. The vials were subjected to shaking for 24 hrs. on a magnetic stirrer. The resultant suspension was then filtered through Whatmann filter paper No.1 and suitably diluted with distilled water. Finally, the samples were analysed by UV-Spectrophotometer (Shimadzu, 1800, Kyoto, Japan) at 257nm.^{8,9}

Estimation of drug content

Solid Dispersion equivalent to 400 mg of Lopinavir were weighed accurately, dissolved in the 50 ml of water and sonicated for 20 minutes. The solution was filtered, diluted suitably. Drug content was analyzed at 257 nm by UV spectrophotometer. The Drug Content was determined using the following equation:^{6,9,10}

$$\text{Drug Content} = \frac{\text{Absorbance of Std. (Cstd.)}}{\text{Absorbance of Tset (Ctest)}} \times 100$$

Dissolution Study

Dissolution rate of Lopinavir and Solid dispersion prepared by different methods were studied by using USP type II (paddle type) dissolution test apparatus (Electrolab, TDT – 08L) in 900 ml of 0.1N HCl with 50 rpm. The temperature was maintained at 37°C throughout the study. Drug and solid dispersion equivalent to 400 mg of Lopinavir was used in each test. Aliquots of 5 ml were withdrawn through a Syringe at different time of intervals. The aliquots were assayed by UV spectrophotometer at 257 nm.⁹⁻¹²

X Ray Diffraction Pattern

Powered XRD were traced employing X ray diffractometer (Bruker D8) for the samples of Lopinavir and solid dispersion of batch CK2, using K-beta filtered K α radiation of wavelength 1.54Å. A voltage of 30kV, a current of 15 mA and receiving slit of a 0.1mm. The samples were analysed over 2 θ scanning range of 20-80° with a time of 2°/minute.

Differential Scanning Calorimetry (DSC)

Thermal characteristics of the Lopinavir, Soluplus and Solid dispersion of batch CK2 were determined by a scanning electron calorimeter (Diamond DSC perkin Elmer). The rate of scanning was 10°C/minute and the scanning temperature range was kept between 0-300°C.

Scanning Electron Microscope (SEM)

The SEM analysis was carried out using a scanning electron microscope (JEOL Model JSM-6390LV). Prior to examination, samples were mounted on an aluminum stub using a double-sided adhesive tape and then making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The

scanning electron microscope was operated at an acceleration voltage of 20 kV.

Preparation and evaluation of Lopinavir immediate release

Tablet and solid dispersion immediate release tablet

Formulation was prepared by using superdisintegrant and other excipients. Immediate release tablets of Pure Lopinavir and optimized batch of selected method of solid dispersion of Lopinavir were formulated by direct compression method by using Karnavati Tablet compression machine. Accurately weighed quantity of Lopinavir and solid dispersion and other excipients were blended together then pass all the materials through 60-mesh screen and compressed into tablets (Table 2 and 3). Prepared tablets were evaluated for Hardness (Monsanto hardness tester), Friability Roche friabilator, Weight variation and dissolution study (Electrolab, TDT – 08L). (Parameters are kept same as per dissolution of solid dispersion).¹³⁻²⁰

RESULTS

FTIR Study

The FT-IR spectra of Lopinavir showed characteristic peaks at 1662.64 cm^{-1} (C=O stretching aliphatic aldehydic group), 3061.03 cm^{-1} (OH stretch hydrogen bonded acidic group), 3396.64 cm^{-1} (NH stretching Amidic group). The spectrum of polymer Soluplus and physical mixture was shown in Figure 1. The spectrum of solid dispersion, which was prepared by kneading method (CK2 batch), was shown the peak at 3396 cm^{-1} due to the intermolecular hydrogen bonding. A number of indistinguishable peaks was shown in the region of 3026–3450 cm^{-1} in the solid dispersion which was also present in the crystalline drug spectra which indicate that the no interaction occurred in between Lopinavir and Soluplus. The results revealed no considerable changes in the IR peaks of Lopinavir, when mixed with Soluplus. These observations indicated the compatibility of Soluplus with Lopinavir.

Solubility study

Table 4 represents the saturation solubility of the pure drug and the solid dispersion of all batches, which was prepared by three different methods by taking various ratios of drug and polymer. Based on solubility studies solvent kneading method and batch CK 2 was selected for formulation of immediate release tablet because they showed highest increase in the solubility of drug in aqueous solution as compare to pure drug and all other batches. The Solvent Kneading method, CK 2 batch (Lopinavir: Soluplus is 1:1) was shown 2.173 folds increase in the solubility.

Drug content

The Drug content profile from the solid dispersion prepared by Physical mixture, Solvent evaporation and Kneading method was mentioned in the Table 5. All the values obtained were expressed in percentage. From the three methods, Solid dispersion prepared by kneading method in 1:1 ratio shown 75.88 percentage drug content

Dissolution Study

Dissolution profiles of the Lopinavir and solid dispersions prepared by Physical mixture, Solvent evaporation and Solvent kneading method was represented in Table 6. It proves that the solid dispersion technique has improved the dissolution rate of all batches as compare to pure Lopinavir to great extent. In addition, Figure 2 represents the dissolution profiles were plotted as the percentage of dissolution from the pure drug and solid dispersions of optimized three batches (AP2, BS2 and CK2). All values expressed in percentage cumulative drug release.

From the dissolution study, it is clearly observed that the dissolution rate of Lopinavir is low because of only 28.5% of drug dissolved in 60 minutes and all solid dispersions batches shows significantly enhanced dissolution rate of 36-64% in 60 minutes. Highest improvement in a dissolution rate was observed in solid dispersion batch CK2 that is prepared by solvent kneading method (1:1 ratio) with corresponding to 60 minutes.

X-Ray Diffraction (XRD) Studies

The XRD pattern of pure drug Lopinavir shown several diffraction peaks at $2\theta = 21.58, 22.73, 26.34, 28.13, 29.77, 31.14$ and 34.44 deg. indicating the crystalline nature of the drug. The XRD of solid dispersion of Lopinavir - Soluplus does not show any diffraction Peaks. It corresponds to the amorphous nature of solid dispersion. (Figure 3 and 4)

Differential Scanning Calorimetry (DSC) studies

In DSC, samples were heated at a constant heating rate at 10°C and the amount of energy required is detected. Pure Lopinavir thermogram showed an endothermic peak at 98.48°C . These indicate that, crystallinity nature of Lopinavir. Soluplus thermogram showed endothermic peak at 76.87°C . (Figure 5)

Solid dispersion thermogram showed endothermic peak at 74.85°C . (Figure 5) In solid dispersion thermogram there was no endothermic peak at 98.48°C , it seems to be polymer get melt and drug particles dissolved in the Soluplus. Crystallinity reduction occurs after the reduction in thermal temperature and melting point. When crystallinity decreases, crystalline nature converted to amorphous which results solubility enhancement.^[103]

SEM Analysis Study (Scanning Electron Microscope)

In order to determine the crystal shape and crystal size of solid dispersion, SEM analysis of the samples were performed. SEM photomicrographs obtained for Lopinavir and Solid dispersion was shown in Figure 6 and 7.

From the photomicrogram of the pure Lopinavir it was found that the nature of pure Lopinavir was irregular crystalline. From the photomicrogram of Solid dispersion was shown dispersion was uniform and drug particles are entrapped within the carrier. It was also confirming by the XRD and DSC analysis.

Tablet preparation and evaluation

To formulate Tablet of Solid dispersion CK2 batch of Solvent Kneading Method was selected on the basis of its Solubility and dissolution performance. Super-disintegrants accelerate disintegration of tablets by virtue of their ability to absorb a large amount of water when exposed to an aqueous condition. The absorption of water results in breaking of tablets and therefore faster disintegration takes place. All the tablets were prepared under similar conditions. Weight variation test of both batches tablet met the requirement for weight variation test according to USP 32 (more than 2 tablets exceeded the official limit of 5% for the $\pm$ percent difference from the mean).

The disintegration time for the tablets correlated well with their hardness (the greater the hardness of the tablet the longer was the time needed to achieve disintegration.) Disintegration time of prepared immediate release tablets was in the range of 33 to 42 seconds. Hardness of tablets prepared by direct compression was 3.8 ± 0.022 to 4.2 ± 0.042 kg/cm². The friability of all Formulations was found to be

less than 1.0 % and hence the tablets with lower friability may not break during handling on machines.

Dissolution profile of Lopinavir tablet and solid dispersion tablet was represented in Table 7. It is evident that the solid dispersion tablet has improved the dissolution rate as compared to Lopinavir tablet to great extent. In addition, Figure 8 represents the dissolution rate profiles were plotted as the percentage of dissolution rate from Lopinavir tablet and solid dispersion tablet. All the values obtained were expressed in percentage cumulative drug release. From the dissolution study, it was clearly observed that, the dissolution rate of Lopinavir tablet was low because of only 32.062% of drug dissolved in 60 minutes and solid dispersion tablet was shown enhanced dissolution rate of 70.985% in 60 minutes.

DISCUSSION

Solubility data demonstrated that, solubility of Lopinavir increases with Soluplus which acts as solubility enhancer polymer. Solubility of solid dispersion was higher in solvent evaporation method as compared to other two methods i.e. Physical method and Solvent kneading method.

The dissolution curves of all batches and pure Lopinavir were presented in figure 2. The release rate profiles were plotted as percentage release of Lopinavir from Solid dispersion from Pure Lopinavir, Physical mixture, Solvent Evaporation and Solvent Kneading method batches versus Time (60 minutes).

It was evident that the rate of dissolution of pure Lopinavir was slow with respective time 60 minutes, while dissolution rate of Physical mixture batch was appeared faster than pure Lopinavir. However it was much lower than of the release of drug from Solvent Kneading method.

XRD of Lopinavir Shows Characteristics Peaks at different angles and indicates that the Lopinavir present in a crystalline form. Results of XRD of Solid dispersion indicate that conversion of Lopinavir Crystalline to amorphous form.

XRD data revealed that the typical pure drug show diffraction peaks due to its Crystalline nature and solid dispersion does not show any type of diffraction peaks due to its amorphous nature. This finding again confirming from the DSC thermogram, it shows an endothermic peak of Lopinavir at 98.48°C . These indicate that, crystallinity nature of Lopinavir and which require the more thermal energy to break the crystal and shows peak at higher temperature. Whereas Solid Dispersion thermogram showed an endothermic peak at 74.85°C which indicate that the amorphous nature of Dispersion which require thermal energy is less than Crystal form so shows peak at lower temperature.

Decrease in the crystallinity of the drug and polymer contribute to enhancement of dissolution of the drug.

CONCLUSION

From the above study, it is summarized and concluded that the SD was prepared successfully by using three methods (physical, solvent evaporation and solvent kneading method). From these, solvent kneading method is more suitable and shows highest results for saturation solubility, drug content, and dissolution rate as compared to Physical method and Solvent evaporation method. Results indicated that used preparation method was promising approach for the solubility, dissolution rate enhancement of Lopinavir and can be formulated as a tablet solid dosage form development for oral use in order to commercialize.

Table 1: Drug-Carrier Formulation in various ratios

Code	Method	Drug-Polymer ratio	Formulation Code
A	Physical Mixture	1:0.5	AP1
		1:1	AP2
		1:2	AP3
B	Solvent Evaporation	1:0.5	BS1
		1:1	BS2
		1:2	BS3
C	Kneading Method	1:0.5	CK1
		1:1	CK2
		1:2	CK3

Table 2: Ingredients used for Pure Lopinavir tablet

Ingredients	Used as	Quantity taken
Lopinavir	API	400
Mannitol (10%)	Diluents/binder	50
Sodium starch glycolate (5%)	Superdisintegrant	25
Magnesium Stearate (2%)	Lubricants	10
Talc (2%)	Glidants	10
Total		495 mg

Table 3: Ingredients used for prepared Solid dispersion tablet

Ingredients	Used as	Quantity taken
Solid dispersion	API	800
Mannitol (10%)	Diluents/binder	95
Sodium starch glycolate (4%)	Superdisintegrant	38
Magnesium Stearate (1%)	Lubricants	9
Talc (1%)	Glidants	9
Total		951 mg

Table 4: Solubility of Pure Lopinavir and Solid Dispersion Batches

Code	Drug:carrier Ratio	Solubility (µg/ml)	Increase in Solubility (Folds)
LOPR	Pure Lopinavir	0.53	--
AP-1	1:0.5	0.897	1.692
AP-2	1:1	1.092	2.060
AP-3	1:2	1.054	1.988
BS-1	1:0.5	0.794	1.498
BS-2	1:1	1.015	1.915
BS-3	1:2	0.954	1.800
CK-1	1:0.5	0.614	1.158
CK-2	1:1	1.152	2.173
CK-3	1:2	1.120	2.113

Table 5: Drug content of solid dispersion

Formulation code	Drug:carrier ratio	Percentage Drug content
AP-1	1:0.5	47.56
AP-2	1:1	52.46
AP-3	1:2	48.92
BS-1	1:0.5	59.63
BS-2	1:1	55.01
BS-3	1:2	63.48
CK-1	1:0.5	59.69
CK-2	1:1	75.88
CK-3	1:2	69.25

Table 6: Dissolution study of Pure Lopinavir and Solid Dispersion

Time (min)	Percentage cumulative Drug release (% cdr)			
	Pure Drug	Physical Mixture	Solvent Evaporation	Solvent Kneading
10	9.6	12.3	15.3	29.4
20	14.4	20.7	21.9	41.1
30	17.7	25.5	29.4	45.9
40	20.4	29.4	33.6	56.1
50	22.8	34.5	36.9	60.3
60	28.5	36.3	40.8	64.5

Table 7: Dissolution study of Pure Lopinavir and of Solid dispersion tablet

Time (min)	(% cdr) of Lopinavir tablet	(% cdr) of Solid dispersion tablet
10	8.8525	33.049
20	12.148	38.839
30	18.707	49.972
40	22.351	57.33
50	26.605	65.316
60	32.062	70.985

Figure 1: FTIR Spectra: (A) Lopinavir, (B) Soluplus, (C) Physical Mixture, (D) Solid Dispersion

Figure 2: Drug Release rate for Pure Lopinavir and solid dispersions

Figure 3: XRD image of pure Lopinavir

Figure 4: XRD image of Solid dispersion

Figure 5: DSC of A. Lopinavir, B. Soluplus and C. Solid dispersion

Figure 6: SEM analysis of Pure Lopinavir

Figure 7: SEM analysis of Solid dispersion

Figure 8: Dissolution study of Lopinavir and solid dispersion tablet

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