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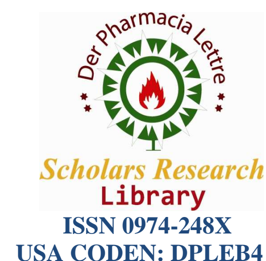

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Designing of Ritonavir Solid Dispersion through Spray Drying

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ABSTRACT

In present study, solid dispersion (SD) of ritonavir was prepared to enhance its aqueous solubility. The SD was prepared by using polyvinyl pyrrolidone vinyl acetate as a carrier with different drug polymer ratios using spray drying technique. The formulation was characterized using differential *scanning calorimetry (DSC), X-ray diffraction (XRD), in-vitro dissolution and in-vivo absorption studies. Intrinsic dissolution rate study was performed to find the impact of increased solubility on the dissolution rate. DSC and XRD analysis demonstrated the conversion of ritonavir to amorphous form. SD showed 95% release in 25 minutes as compared to 20% of drug release in 60 minute form physical mixture. in-vivo study result indicated that SD exhibited significant increase in area under concentration with time (AUC) and maximum concentration (C_{max}).*

Key words: Solid dispersion, spray drying, ritonavir.

INTRODUCTION

Oral route is a desirable route for drug administration due to convenience and good patient compliance. When a drug is to be orally absorbed, it must be dissolved in gastric and/ or intestinal fluid. However, a number of promising drugs generated by technological innovations are poorly water soluble and it is difficult to adopt them as candidates for new drug though they may exhibit good pharmacological activity. This poor water solubility leading to low dissolution rate in the gastrointestinal fluid often lead to insufficient bioavailability.

Different techniques those have been used to enhance the dissolution rate in the case of poorly soluble drugs, includes the use of surfactants, polymorphism, drug micronization and solid dispersion. Solid dispersion is widely used as it can produce a solid dosage form having the active in an amorphous state or in a molecular dispersion state. The improvement of dissolution from solid dispersion is attributed to drug particle size reduction, solublization effect of the carrier, generation of amorphous state and specific molecular interaction between the drug and carrier. Several techniques for manufacturing of solid dispersions like solvent evaporation and melting method have been described in the literature. In terms of solvent evaporation methods

spray drying technique shows to be an efficient technique for production with significant improvement in dissolution properties, may be due to their potential of conversion of crystalline state of drug to amorphous state, one step process, production of spherical particles with high surface area etc.

Protease inhibitor is one of the important classes for the treatment of AIDS, since drugs of this category are independently act on the glycoprotein synthesis inhibiting growth of human immunodeficiency virus. Among various examples, ritonavir is a potential candidate which acts as protease inhibitor as well as substrate for efflux pump, which inhibits elimination of other drug candidates through this pump when used in association. However, the applicability of ritonavir itself is limited due to its extremely low solubility in gastric fluid. Its solubility has been reported to be greatly improved i.e. when solid dispersion with PEG 8000 were employed, (100% release of drug was obtained when the drug-polymer was in the ratio 1:9). But this increased solubility has a problem of outsize increase in total weight of unit product. It has been previously reported that solid dispersion prepared with polyvinyl pyrrolidone vinyl acetate has significantly improved solubility of different drug molecules due to its capability of forming solid drug solution, ability to stabilize for longer duration, inhibition of drug recrystallization, low toxicity, low cost, rapid solidification etc.

As evident form number of papers published, the possibility of applying polyvinyl pyrrolidone vinyl acetate to ritonavir has not been reported so far. In this paper, ritonavir was assessed for formulation development using polyvinyl pyrrolidone vinyl acetate as a carrier material employing spray drying technology.

The formulations of ritonavir-polyvinyl pyrrolidone vinyl acetate from spray dried solid dispersions were compared with pure drug fallowing the criteria of intrinsic dissolution rate, dissolution rate and solid state characterizations by differential scanning calorimetry, Fourier transform infra red spectroscopy and X-ray diffractometer. The absorbed amount of ritonavir after oral administration of solid dispersion with polyvinyl pyrrolidone vinyl acetate to *albino wistar rats* was judged against the pure drug.

MATERIALS AND METHODS

2.1. Materials:

Ritonavir was provided courtesy Matrix Laboratories Ltd., Nashik, India. Polyvinyl pyrrolidone vinyl acetate was a kind gift sample from BASF India Ltd., Navi Mumbai, India. All other chemicals were locally procured and solvents of Merck grade were used.

2.2. Preparation of physical mixture:

Physical mixtures were prepared by grinding ritonavir and polyvinyl pyrrolidone vinyl acetate in a mortar and pestle for 10 minutes. The weight ratio of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 of the ritonavir: polyvinyl pyrrolidone vinyl acetate were obtained. This mixture was screened through sieve 80 mesh to yield a product having particle size in the range of 150 µm.

2.3. Preparation of spray dried powder:

Solid dispersions of ritonavir and polyvinyl pyrrolidone vinyl acetate in different ratios were prepared using spray drying method. For this 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 weight ratios of ritonavir: polyvinyl pyrrolidone vinyl acetate were dissolved in dichloromethane to represent 5% w/v of the polymer solution. This solution was fed to spray dryer (Labultima, Jay Instruments and Systems Pvt. Ltd., Mumbai, India) at a rate 1 ml/ minute and sprayed into the drying

chamber from a nozzle with a diameter 400 µm at a pressure of 0.15 MPa. The inlet and outlet temperatures of drying chamber were maintained at 80° C and 50° C respectively. The powder obtained was further dried in a vacuum dessicator for 24 hrs before their physicochemical parameters were tested.

Details of batch information are given below (Table 1):

Table 1: Batch information of weight ratios for ritonavir: polyvinyl pyrrolidone vinyl acetate.

Weight ratio of ritonavir: polyvinyl pyrrolidone vinyl acetate	Batch Number	
	Physical mixture	Solid dispersion
1:1	PM1	SD1
1:2	PM2	SD2
1:3	PM3	SD3
1:4	PM4	SD4
1:5	PM5	SD5
1:6	PM6	SD6

2.4. Differential scanning calorimetry (DSC):

A differential scanning calorimeter (DSC 821^e, Mettler Toledo, Switzerland) equipped with a refrigerated cooling system was used to determine the degree of drug crystallinity in the formulations. About 2-4 mg of sample in an aluminium pan was heated at a scanning rate of 10° C per minute form a temperature 30° C to 150° C under nitrogen gas flow. Calibration of temperature and heat flow was carried out with Indium. The heat of fusion of the formulations was measured. The ratio of fusion enthalpies form formulation was used to calculate the extent of relative drug crystallinity.

2.5. X-ray diffraction (XRD):

Philips PW3040 X-ray diffractometer (PANanytical Inc., Netherlands) was controlled by X'pert software with Cu Kα radiation generated form a copper source operating at power level of 40KV and 40 mA. Sample discs were used for XRD analysis and scanned over a range between 7°- 25° at step size of 0.02°/step and the a rate of 0.4 second/step.

2.6. FT-IR spectroscopy (FT-IR):

FT-IR spectra of the preparations were obtained using FT-IR IR AFFINITY-1 (Toshvin Analytical, Shimadzu, Japan). The sample was dispersed in dry potassium bromide, ground well in mortar and pestle fallowed by disc preparation. The disc was placed in the FT-IR sample holder and IR spectra, in absorbance mode, were obtained in the spectral region 4000 cm^{-1} to 400 cm^{-1} using resolution of 4 cm^{-1} and 40 scans.

2.7. Tableting:

All formulations were compressed into flat and round tablets using a compression machine (Tablet machine single punch, National Engg. Works, Mumbai, India). Tablets containing weight equivalent to 100 mg of ritonavir were prepared corresponding to hardness ~ 5 kg/cm^2. The formulations were compression friendly, thus no exciepient were employed in the process. The diameter was 13 mm and weight was varying with formulations.

2.8. *in-vitro* study:

in-vitro release profile for pure drug, physical mixture and solid dispersion were performed using USP type 1 dissolution apparatus (Electrolab TDT-08L Dissolution Tester USP, Mumbai, India).

The test was carried out at 37 ± 0.5° C at a rotation speed of 100 rpm for a period of 60 minutes using 900 ml of 0.1 N HCl as dissolution medium.

Intrinsic dissolution rate test was carried out to identify the change in drug solublization. For this, tablets obtained form the compression were brought in contact of molten beeswax in such a way that one face could remain uncovered. Thus one face and side of tablet were coated with the hydrophobic wax. The area of surface uncoated was 1.326 cm^2. This surface was available to be in contact with dissolution medium for intrinsic dissolution rate study. The cumulative amount dissolved per cm^2 was plotted against time. The slope of linear region ($R^2 \geq 0.95$) was taken as intrinsic dissolution rate.

For dissolution test, ritonavir, physical mixture and solid dispersion tablets were transferred to dissolution medium for the measurement. The % dissolved of ritonavir was plotted against time. Experimental conditions for intrinsic dissolution rate as mentioned above and dissolution rate were maintained same.

For both Intrinsic dissolution rate and dissolution rate samples of 5 ml were withdrawn at predetermined time points and the amount withdrawn was immediately replaced with fresh dissolution medium maintain at the same temperature. The samples drawn were filtered through 0.45 µm filter and concentration of ritonavir determined using HPLC (Jasco HPLC 900, Japan).

2.9. *in-vivo* study:
The animal experiment protocol was approved by institutional ethical committee. *albino wistar* rats (10 weeks, 150-160 gm. Bharat Serum, Mumbai, India) were used for *in-vivo* studies. The animals were divided into two groups each having 6 in numbers, fasted for 12 hr. before the study. Pure drug and solid dispersion were orally administered (10 mg of ritonavir/kg) separately to each group using an oral dosing syringe after dispersing in 1ml distilled water. Food and water was not given during the following 6 hour period of test after administration of the dose. For the pharmacokinetic analysis, blood samples of about 600 µl were collected from tail vein at time points 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6 hours after administration and kept in heparin tubes. Blood samples were centrifuged at 10,000 rpm for 10 min at room temp to get plasma which were frozen (–20° C) immediately and maintained at this temperature until analyzed. A cross-over experiment was performed two weeks later, after the first administration.

2.10. HPLC analysis:
Analysis was performed using HPLC (Jasco HPLC 900, Japan) equipped with 4.6 X 250 mm Qualisil® BDS C-18 column. The analytical operational conditions were, the mobile phase: 60 volumes of acetonitrile and 40 volumes of a buffer solution prepared by dissolving 3.4 g of sodium acetate and 0.94 g of sodium hexane sulphonate in 1000 ml of water adjusting the pH to 4.0 with HCl, the flow rate: 1 ml/min., the wavelength: 239 nm and injection volume: 100 µl.

Valacyclovir was used as internal standard for *in-vivo* study. 100 µl of methanol containing Valacyclovir (25 µg /ml) was added to the 100 µl of the plasma. Subsequently 2 ml of acetonitrile was added, the mixture vortexes and centrifuged at 10,000 rpm for 10 minute. to separate off plasma proteins. The organic phase was transferred to a glass tube and evaporated to dryness using a stream of nitrogen gas at 37° C. The residue was dissolved in 200 µl mobile phase and ritonavir was determined by HPLC. The concentration of ritonavir in the plasma was calculated using regression equation.

RESULTS AND DISCUSSION

3.1. Intrinsic dissolution rate (IDR):

Impact of increased solubility on the dissolution rate was investigated through intrinsic dissolution rate studies. The latter is a parameter which could be easily used to identify the change in drug solublization rate and it is expected to correlate more closely to *in-vivo* dissolution dynamics of drugs than solubility alone. The intrinsic dissolution rate studies were performed in 0.1 N HCl for 60 min. Experimental observations for intrinsic dissolution rate of pure drug, physical mixtures and solid dispersions (Fig. 1) are provided in (Table 2).

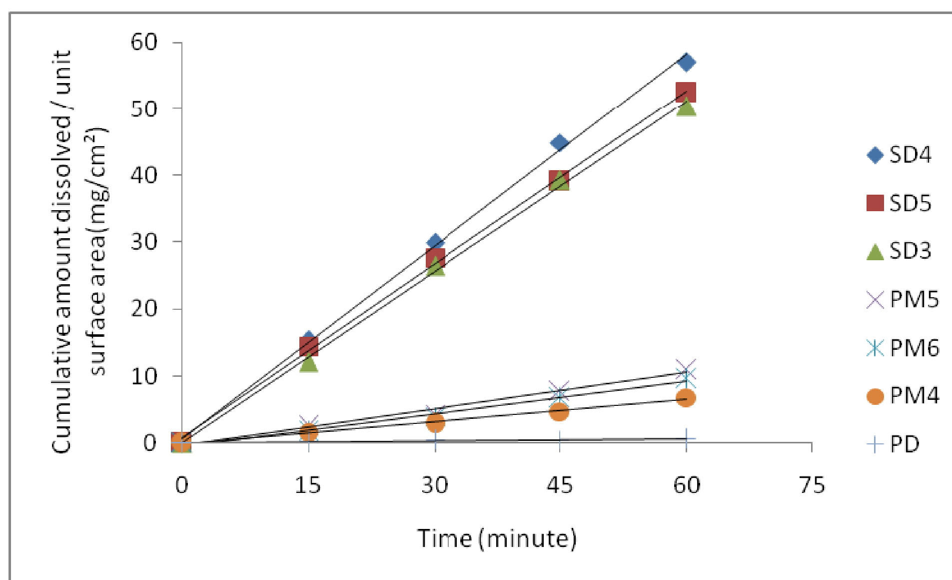

Fig 1 Intrinsic dissolution rate determination

Table 2: Intrinsic dissolution rate of pure drug, physical mixtures and solid dispersions

Batch Number	Intrinsic Dissolution Rate
PD	0.009 mg/min/cm²
PM4	0.169 mg/min/cm²
PM5	0.186 mg/min/cm²
PM6	0.162 mg/min/cm²
SD3	0.754 mg/min/cm²
SD4	0.937 mg/min/cm²
SD5	0.793 /min/cm²

The release rate of ritonavir form physical mixture and solid dispersion varied with the changing ratios of ritonavir and polyvinyl pyrrolidone vinyl acetate respectively. It was found that the influence on the dissolution of ritonavir increased with the ritonavir and polyvinyl pyrrolidone vinyl acetate ratio. In the samples with lower ritonavir and polyvinyl pyrrolidone vinyl acetate ratio, the dissolution surface might become depleted of polyvinyl pyrrolidone vinyl acetate with time causing subsequent reduction in the concentration of this polymer in diffusion layer and hence affect formers solublization. This had resulted in decrease in intrinsic dissolution rate. As the ratio of ritonavir: polyvinyl pyrrolidone vinyl acetate reached 1:4 for solid dispersion and 1:5 for physical mixture, maximum release rate of ritonavir was evident.

Decrease in intrinsic dissolution rate was observed at higher concentration of polymer as shown form the batches SD5 and PM6. It has been suggested that it might be caused by the leaching out of the carrier during dissolution which could form a layer of concentrated solution around the ritonavir particles. Thus, the migration of released ritonavir to the bulk of dissolution medium was slowed down.

Comparative intrinsic dissolution rate of PD (IDR= 0.009 mg/min/cm²), PM5 (IDR= 0.188 mg/min/cm²) and SD4 (IDR= 0.937 mg/min/cm²) made it clear that these exhibited a linear relationship between the amount of ritonavir dissolved and the time elapsed. It was found that the release rate of ritonavir for the physical mixture was significantly higher compared to the pure drug. Further, the solid dispersion resulted in remarkable increase in release rate of ritonavir compared to that of physical mixture.

The low intrinsic dissolution rate for pure drug was likely due to the presence of intermolecular hydrogen bonding in the ritonavir molecules. Such bonding is difficult to break during dissolution leads to availability of low surface area for dissolution resulting into slow intrinsic dissolution rate. The release rate of ritonavir from all the batches of physical mixture was significantly improved. This phenomenon could be ascribed to the solublization effect of polyvinyl pyrrolidone vinyl acetate. Ritonavir particles were in close contact or adhered to the polymer particles as a result of mixing and grinding process. When a powder mixture was brought in contact with water the polymer particles hydrated rapidly to form a concentrated viscous solution that probably solublizes the adjacent ritonavir particles and sufficiently immobilized to prevent aggregation. Subsequently the ritonavir was released as the polymer dissolved into the medium.

These observations enabled to choose the batches which have shown maximum intrinsic dissolution rate. Batch PM5 of physical mixtures and SD4 of solid dispersions were chosen for further dissolution rate and bioavailability studies.

3.2. Differential scanning calorimetry (DSC):

For each system representative DSC curves of the samples processed at 30° C to 150° C are shown in Fig. 2. Ritonavir showed a sharp endothermic peak at 122° C, representing its melting point. During the scanning of polyvinyl pyrrolidone vinyl acetate, a broad endotherm at 80° C was observed indicating amorphous nature of the polymer. Thermo gram of solid dispersion also showed similar broad endotherm, a broad peak was observed between 60° C to 80° C, but no endotherm was observed around the melting point of ritonavir indicating that ritonavir was available in its amorphous state. The physical mixture showed endotherm corresponding to melting point of ritonavir hinting presence of crystallinity. Moreover, beside the endothermic peak of ritonavir, the thermo gram of PM5 showed an endothermic zone in the range of 112° C to 127° C which could be ascribed to eutectic nature of formulation.

3.3. X-ray diffraction (XRD):

The influence of polyvinyl pyrrolidone vinyl acetate in the possible phase transformation in PM5 and SD4 was investigated through X-ray diffraction (Fig. 3). The X-ray diffraction pattern for pure drug showed numerous strong distinctive peaks at ~ 9°, 10°, 14°, 16°, 18°, 20°, 22° at 2 theta indicating high crystalline nature. The typical peak intensities in case of physical mixture were lower than those of pure drug indicating partial crystallinity in this system. This hinted that, physical mixture were dispersion of drug at amorphous as well as crystalline level in the carrier polymer, polyvinyl pyrrolidone vinyl acetate. Finally, X-ray diffraction pattern of solid

dispersion showed no diffraction peaks, indicating that this was practically composed of amorphous drug.

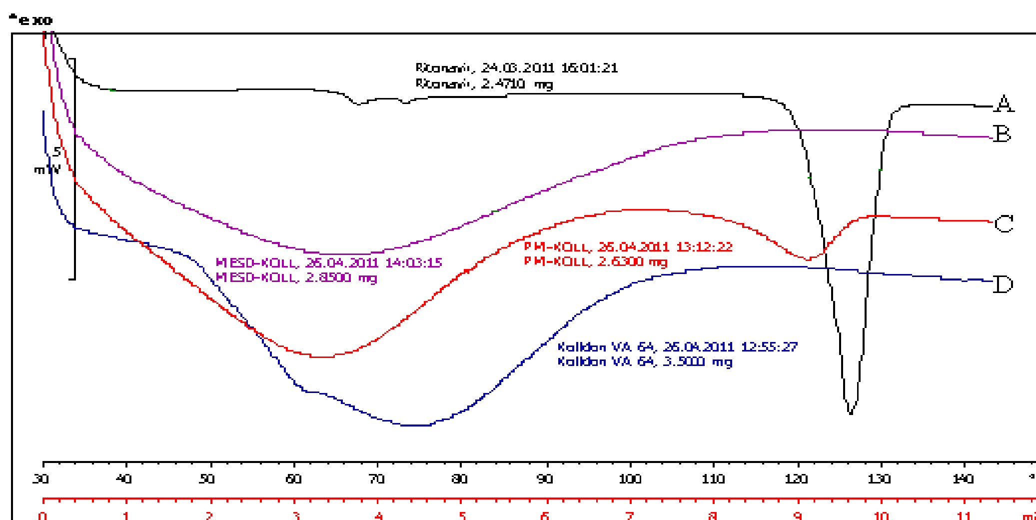

Fig 2 DSC thermo grams of the (A) PD, (B) SD4, (C) PM5 and (D) polyvinyl pyrrolidone vinyl acetate.

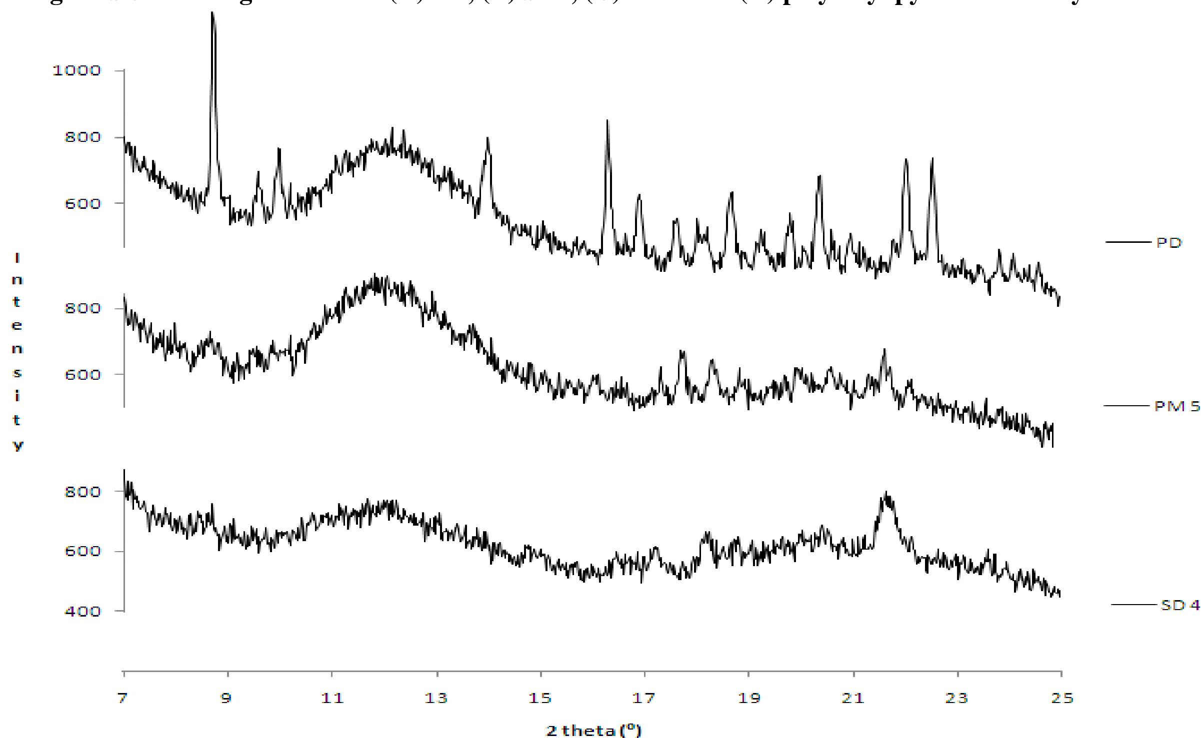

Fig 3 X-ray diffractograms of PD, PM5 and SD4 indicating amorphous nature of solid dispersion.

In conclusion, the crystallinity of ritonavir proved to be growing weaker and weaker as the system moved form pure drug to physical mixture to solid dispersion. Thus, during manufacturing of solid dispersion the crystallinity of ritonavir had vanished or minimized to negligible. These findings were consistent with DSC results.

3.4. FT-IR spectroscopy (FT-IR):

The FT-IR experimental observation (Fig. 4) showed spectroscopic intensity at the 2872.44 cm^{-1}, 2966.52 cm^{-1}, 3024.38 cm^{-1}, 3057.17 cm^{-1} and 3159.40 cm^{-1} consulted that the hydrogen bonding

was essentially present in ritonavir-polyvinyl pyrrolidone vinyl acetate. Pure drug showed characteristic absorption bands at 2870.08 cm^{-1} and 2962.66 cm^{-1} assigned as hydrogen bonding between ritonavir molecules which could not be broken by aqueous fluid during dissolution resulting into low solubility. Physical mixture showed a characteristic absorption band at 2870.08 cm^{-1} and 2962.66 cm^{-1} assigned as hydrogen bonding between ritonavir molecules not essentially lost. This supported the presence of crystalline nature of ritonavir in the polyvinyl pyrrolidone vinyl acetate.

The interactions of hydrogen bonding between ritonavir-polyvinyl pyrrolidone vinyl acetate molecules were investigated through FT-IR. As expected form DSC and XRD studies, solid dispersion showed disruption of intermolecular hydrogen bonds in among ritonavir molecules. This disruption has been replaced by hydrogen bonding between ritonavir- polyvinyl pyrrolidone vinyl acetate molecules.

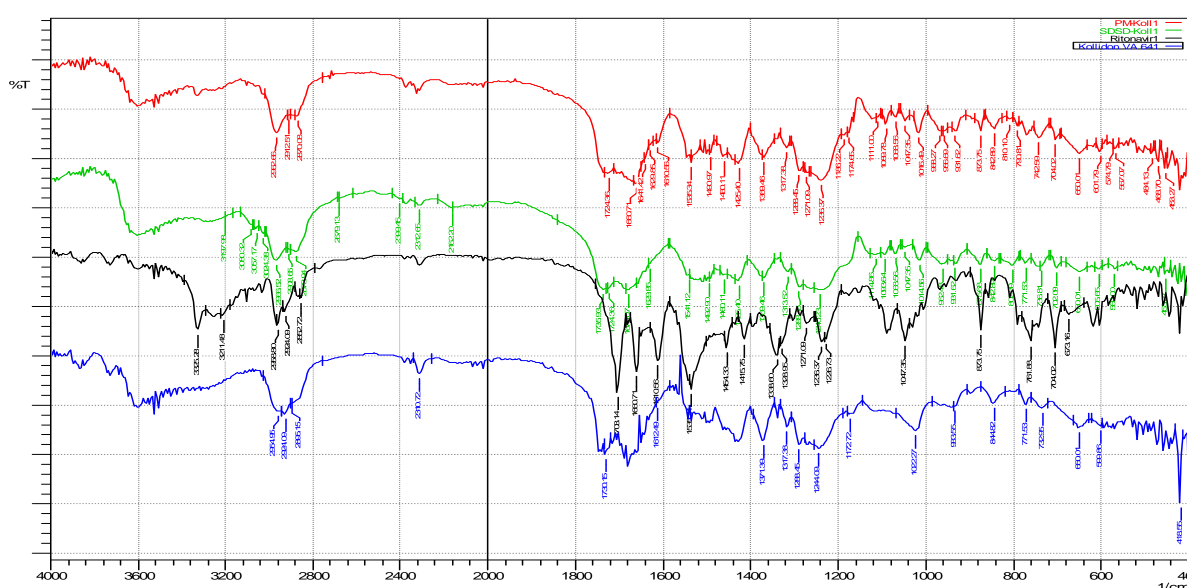

Fig 4 FT-IR spectroscopic absorption peak of PM5, SD4, PD and polymer (from top to bottom) showing intermolecular hydrogen bonding.

3.5. Dissolution rate (DR):

Dissolution studies were performed under sink conditions using 0.1 N HCl. Physical mixture and solid dispersion tablets of ritonavir were tested for the dissolution properties and compared with the pure drug. The results as dissolution profiles are shown in the Fig. 5, shows 95% dissolution of SD4 in 25 minutes while only 20 releases occurred in PM5 in 60 minutes.

It could be noted form these values that release rate of ritonavir was higher form the solid dispersion compared with the pure drug and physical mixture. Solid dispersion provided higher in initial dissolution rate than pure drug. This high initial rate for the solid dispersion might be attributed to the hydrogen bonding between ritonavir and polyvinyl pyrrolidone vinyl acetate, which breaks relatively easily during dissolution compared to the ritonavir intermolecular bonding present in the pure drug.

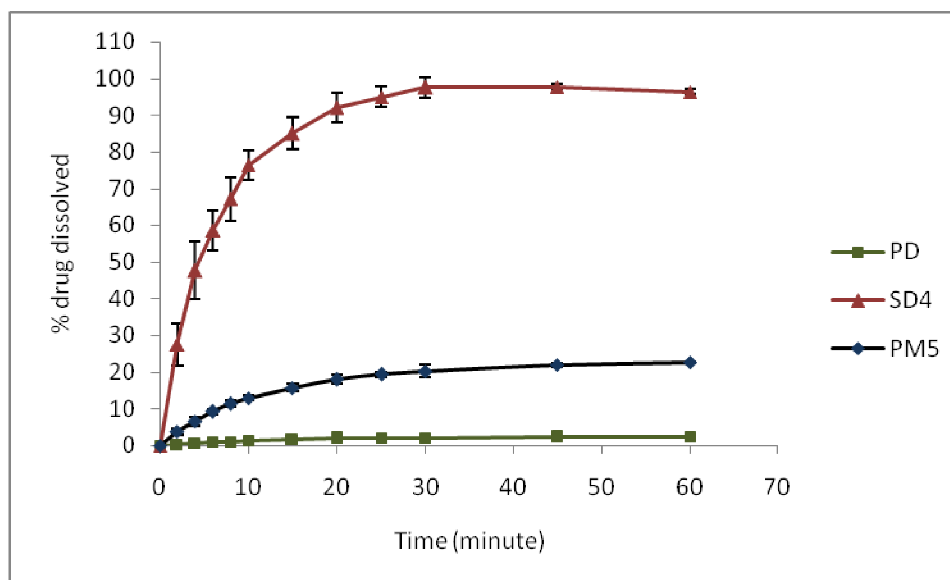

Fig 5 Dissolution profiles.

For the physical mixture, mechanism of partial amorphization as well as wetting effect of polyvinyl pyrrolidone vinyl acetate might have operated and sequentially making and makes dissolution rate higher than the pure drug. In the case of solid dispersion several factors including particle size reduction, wetting through intimate contact between ritonavir and polyvinyl pyrrolidone vinyl acetate and amorphization impacted significantly on dissolution rate which produced higher release rate.

3.6. Bioavailability (BA):

The pure drug and solid dispersion were compared through the *in-vivo* study. The plasma concentration profiles of ritonavir after oral dosing (10 mg/kg body weight) to the overnight fasted *albino wistar* rats are shown in the Fig. 6, indicating rate of absorption form the solid dispersion to be rapid and resulting plasma concentrations significantly higher than pure drug, indicating that dissolution was indeed the rate limiting step for the absorption of ritonavir form pure drug. The C_{max} value of solid dispersion was significantly higher. This results suggested that the absorption rate of solid dispersion was notably higher than that of pure drug. SD resulted in much higher AUC compared with PD which was reflected by AUC. Results indicates that AUC $_{(t = 8 \text{ hours})}$ of SD was 59.62 (µg/ml hour) compared with that PD which was 8.08 (µg/ml hour)

Table 2 Bioavailability parameters of PD and SD4 in *albino wistar* rats

Batch Number	C_{max} (µg/ml)	AUC $_{t = 0\text{-}8 \text{ hours}}$ (µg/ml hour)
PD	3. 18	8.083957
SD4	14.28	59.62133

Crossover methodology was put into force to rule out the influence of individual difference in *albino wistar* rats. It was revealed through *in-vitro* study that the drug release from solid dispersion was higher than that of pure drug. The amount of water available would also influence on the dissolution at any given dissolution site. The dissolution duration for the entire dose is longer than that of residence time of the drug in the absorption region of upper GIT reflecting in low bioavailability for pure drug whereas solid dispersion showed significant increase in apparent solubility and hence the bioavailability.

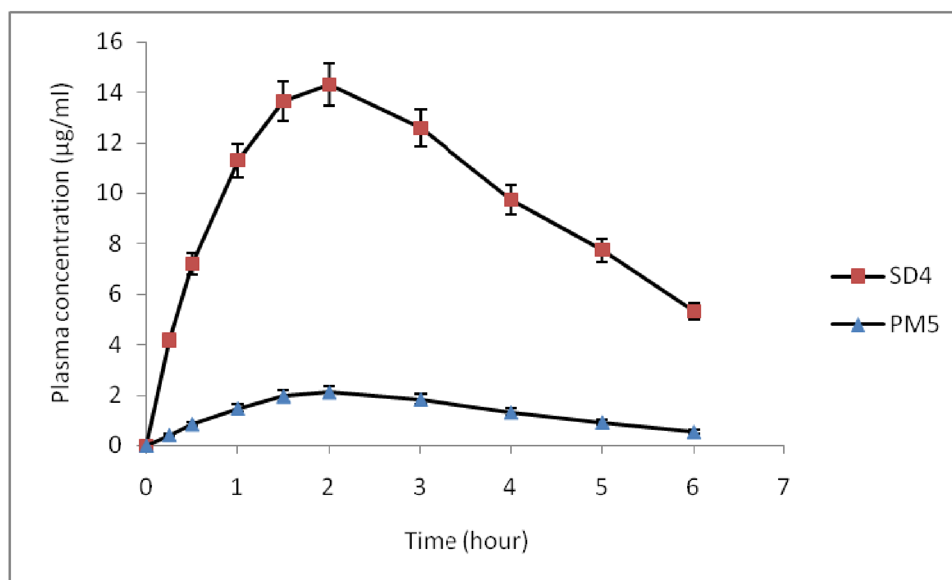

Fig 6 Mean plasma concentration-time profiles of ritonavir form PD and SD4.

CONCLUSION

The *in-vitro* dissolution properties of ritonavir and *in-vivo* studies of plasma concentration of ritonavir in *albino wistar* rats after oral administration form solid dispersion were estimated. Solid dispersion of ritonavir showed marked improvement in bioavailability compared to physical mixture and pure drug.

When solid dispersion of ritonavir-polyvinyl pyrrolidone vinyl acetate was administered to rats orally, the maximum plasma concentration and AUC of ritonavir significantly increased compared to pure ritonavir. This suggested that solid dispersion of ritonavir-polyvinyl pyrrolidone vinyl acetate could improve both the dissolution rate and bioavailability of a poorly water soluble drug ritonavir.

The DSC, SEM and XRD studies showed that the ritonavir was in an amorphous state in the solid dispersion. This confirmed that the proposed solid dispersion form would be useful to increase the dissolution rate and consequently bioavailability of ritonavir.

It was further the extent of improvement in drug dissolution depended upon the extent of drug loading with low drug loading grater improvement in the dissolution was noted.

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