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ORIGINAL ARTICLE

Complexation approach for fixed dose tablet formulation of lopinavir and ritonavir: an anomalous relationship between stability constant, dissolution rate and saturation solubility

Gaurav Goyal · Pradeep R. Vavia

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Abstract In the present investigation, cyclodextrin complexation process was explored for development of tablet formulation of WHO approved fixed dose combination of lopinavir and ritonavir with reduced tablet size, shorter disintegration time and higher bio-availability in comparison to reference product. In preliminary studies, we found that lopinavir solubility and dissolution rate is poor into the dissolution medium recommended by FDA, whereas ritonavir solubilized fairly into dissolution medium with adequate dissolution rate. Solid-state cyclodextrin complexation technology was used for enhancement of dissolution rate of lopinavir into dissolution medium. Various cyclodextrins were screened by comparison on basis of enhancement of dissolution rate of lopinavir (LPV) and the order was found as gamma cyclodextrin (γ-CD) > hydroxypropyl beta-cyclodextrin (HP-β-CD) > methyl beta-cyclodextrin (M-β-CD) > beta-cyclodextrin (β-CD), with Q_{120} values (i.e. percentage of dissolved drug at 120 min.) were 10.1 for the pure LPV and 56.3, 51.3, 30.3 and 10.3 for LPV/γ-CD, LPV/HP-β-CD, LPV/M-β-CD and LPV/β-CD, respectively. Anomalous results were found between stability constant, dissolution rate and saturation solubility. It was found that cyclodextrin having higher stability constant value with LPV, provides higher saturated solubility of LPV in aqueous media but at slow dissolution rate and vice versa. The γ-CD was selected for complexation with lopinavir in the stoichiometric ratio 1:1.5 M of LPV to γ-CD. Various processes such as kneading method, milling technique, sonication, freeze drying and autoclaving were tried, from which kneading method was found to give best dissolution results. The corresponding solid complexes were characterized by differential scanning calorimetric, X-ray powder diffraction and scanning electron microscopy studies. Based on various studies, the complexation phenomenon between LPV and γ-CD was found to follow non-inclusion behavior. Pharmacokinetic studies were carried out in Sprague-Dawley rats using cross over design with a 3 day wash out period. The bioavailability of lopinavir was found to be enhanced significantly using cyclodextrin complex tablet formulation.

Keywords Lopinavir · Ritonavir · Cyclodextrin · Dissolution rate · Bioavailability · Protease inhibitors

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Introduction

Human Immunodeficiency Virus (HIV) infection and acquired immune deficiency syndrome (AIDS) treatment was revolutionized with the invention of HIV protease inhibitors (PIs) [1]. Various protease inhibitors are approved to be taken in combination regimens for better antiviral efficacy and to avoid resistance of drugs, commonly known as highly active antiretroviral therapy (HAART) [2]. Due to high pill burden, PIs remain an essential component of HAART, especially for treatment-experienced patients [3, 4]. These inhibitors interfere in a replication phase of HIV, resulted in impairing of formation of active functional and structural proteins that are necessary for the maturation of the virus from viral precursor polypeptides [5, 6]. Because of poor oral bioavailability of many of the presently used protease inhibitors, many efforts have been made to develop formulations which can provide better pharmacokinetic characteristics.

One such fixed dosed combination approved by FDA was lopinavir and ritonavir (LPV/r) in soft gel capsules, liquid formulation and tablet dosage form. Ritonavir, below its therapeutic dose is indicated in combination with many other antiretroviral agents as the synergistic enhancer. Ritonavir acts by reducing the liver metabolism and results in higher bioavailability of other potent antiretroviral drugs [7].

LPV/r both belongs to Biopharmaceutics Classification System (BCS) Class 4 i.e. low permeability and low solubility. Soft gel capsules (SGC) formulation was the first approved formulation for LPV/r. SGC were based on encapsulation of LPV/r in liquid oily matrix consist of oleic acid, propylene glycol, sorbitol and castor oil. Due to storage stability problems at ambient conditions and gastrointestinal side effects, SGC were replaced with tablets based on melt extrusion technology. The melt-extrusion technology reduced pill count from 6 SGC (LPV/r; 133.3 mg/33.3 mg) per day to 4 tablets (LPV/r; 200 mg/50 mg) per day [8].

The tablet formulations of LPV/r based on melt extrusion technology has certain associated drawbacks such as difficulty in swallowing due to big tablet size, lag time for action due to high disintegration time and composite method of manufacturing. The objective of the present work was to find the suitable technology addressing the issues related of melt extrusion. In combination, ritonavir is used below the therapeutic dose, as an enhancer for lopinavir bioavailability; therefore the aim of the present work is to device the technology which can provide equal or higher bioavailability of lopinavir in comparison to the reference marketed product based on melt extrusion technology.

In literature, many methods are reported for enhancement of oral bioavailability of antiretroviral drugs, for example, solid dispersion [9], use of grapewine juice [10], micelles and microemulsions [11], sustained bio-adhesive tablet [12], pH sensitive nanoparticles [13], nanopowders [14], cyclodextrin complexation [15] and etc. From these reported methods, we selected cyclodextrin complexation method for our studies because of two reasons; complexation using cyclodextrins is easily scalable and reproducible; moreover cyclodextrins are reported to enhance the solubility as well as permeability of BCS class 4 molecules [16].

Experimental

Materials

The materials used were lopinavir (Batch Lo 0091207, Hetero Drugs, IN), ritonavir (Batch RI 0010208, Hetero Drugs, IN), gamma cyclodextrin (Wacker Chemie AG, IT), hydroxypropyl beta-cyclodextrin (Roquette, FR), methyl beta-cyclodextrin (Roquette, FR) and beta-cyclodextrin (Roquette, FR), polyoxyethylene 10 lauryl ether (POLE, Sigma Aldrich, IN), potassium dihydrogen phosphate GR (KH_2PO_4, Merck, IN), ortho-phosphoric acid GR (Merck, IN), acetonitrile HPLC (Merck, IN), EDTA Dipotassium Salt (Merck, IN), Gum Tragacanth (SD Fine Chemicals, IN) and Sodium Hydroxide (NaOH, Merck, IN). All the materials were used as received without further purification.

HPLC analysis

High-performance liquid chromatography (HPLC) analysis were performed with a Waters HPLC unit Model 515 pump equipped with a Waters 2487 dual wavelength UV detector and a 200 µL loop injection valve. For analysis, Thermo, 25 cm × 4.6 mm, ODS, 5 µm column was eluted with mixture of acetonitrile and 0.03 M KH_2PO_4 solution (56:44), final pH adjusted to 3.24 by O-phosphoric acid. HPLC mobile phase was prepared from HPLC-grade Acetonitrile. A flow rate of 1.0 mL/min was maintained. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions.

Preliminary studies

Phase solubility studies of LPV were carried with each cyclodextrin according to the method reported by Higuchi and Connors [17]. Excess amount of LPV was added to aqueous solutions containing various CDs' respectively in different molar concentrations and stirred for 48 h at constant temperature, 25 °C on orbital shaker. The solutions were filtered through 0.45 micron nylon filter and the filtered solutions were analyzed using validated HPLC method. The apparent 1:1 stability constant K_c was calculated from the straight line of the phase solubility diagram by using the following equation, Eq. 1:

$$K_c = \text{slope}/S_0(1 - \text{slope}) \quad (1)$$

where, K_c is the stability constant (M^{-1}), slope is obtained from the linear relationship between the concentration of drug (y axis) and cyclodextrin (X-axis) and S_0 is drug solubility (M) at 25 °C without cyclodextrin.

Based on the results of phase solubility studies, complexation efficiency of each cyclodextrin with LPV was calculated [18]. Complexation efficiency (CE) is defined as the solubilizing efficiency of CD and is equal to complex to free cyclodextrin concentration ratio; Eq. 2.

$$CE = [D/CD]/[CD] = Slope/(1 - Slope) \quad (2)$$

The saturation solubility studies were conducted in conical flask by shaking excess amount of LPV in 25 mL of 0.382 mM of CDs' used for study, in aqueous solution at 25 ± 2 °C for a time period till saturation was achieved. It was found that for all CDs'; saturation of LPV was achieved within 30 h.

Selection of cyclodextrin

The complex of each cyclodextrin, β-CD, γ-CD, HP-β-CD and M-β-CD was formulated with LPV using kneading method in the ratio of 1:1 on molar basis. Total four complex mixtures were formed. Each complex mixture was passed through 100# sieve. Each sieved complex equivalent to quantity of LPV per tablet was subjected to dissolution studies. The selection was performed on the basis of comparative in vitro dissolution studies, Q_{120} (percentage of dissolved drug at 120 min). The samples were analyzed by HPLC, after filtration through 0.45 micron nylon 6, 6 filter membrane.

Permeation studies

Cyclodextrins are well reported as solubilizing agents as well as permeation enhancers. We conducted permeation studies, to investigate the role of different cyclodextrins on rate of permeation (flux). The protocol for the permeation studies was approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA; Protocol No. UICT/PH/IAEC/0807/11). The investigation was performed by in vitro everted rat intestinal sac model [19]. About 1.4 mL tyrode solution was used as serosal effluent. The mucosal effluent comprised of saturated solution of LPV (3.60×10^{-3} mM) with different cyclodextrins in concentration of 0.4 mM in tyrode solution. At predetermined time intervals the serosal solution was removed and analyzed by developed HPLC method.

Sprague-Dawley rats (weighed 200–250 g, were obtained from Haffkine Institute, Mumbai, IN) were anesthetized with 20% w/v ethyl carbamate solution. A 20 cm segment of jejunum was quickly removed, rinsed with tyrode buffer and everted. This segment was tied at one end with a cotton thread, filled with oxygenated tyrode buffer and then tied at the other end. The resultant large sac was divided into four 5–6 cm sacs by tying at intervals. Each sac contained about 1.4 mL oxygenated tyrode buffer. The rats were then sacrificed by injection of ethyl carbamate into the heart.

Each sac was individually placed in a 15 cm high glass tube containing 10 mL oxygenated mucosal effluent kept in a water bath at 37 ± 2 °C. The entire volume of inside medium was taken at predetermined time point of 1 h and analyzed for LPV concentration using HPLC method and flux was calculated for each cyclodextrin.

Preparation of binary system [20]

Selection of suitable method for complexation of LPV with selected cyclodextrin; γ-CD was performed on the basis of comparative in vitro dissolution studies.

Physical mixture

A physical mixture (PM) was prepared by mixing LPV and γ-CD together in a geometric proportion in 1:1 M ratio and passed through a 100# sieve.

Kneading method

In the kneading method, the physical mixture of LPV and γ-CD in 1:1 M ratio was triturated in a mortar with a small volume of water–methanol (1:1 vol/vol) solution. The thick slurry was kneaded for ~45 min and then dried at 60 °C under vacuum. The dried mass was pulverized and passed through a 100# sieve.

Sonication method

In the sonication method, the aqueous solution of γ-CD was added to the alcoholic solution of LPV in 1:1 M stoichiometry. The resulting mixture was sonicated for 1 h and evaporated under vacuum at 60 °C. The dried mass was pulverized and passed through a 100# sieve.

Freeze-drying method

In the freeze-drying method, the required 1:1 M quantity of LPV was dispersed in an aqueous solution of γ-CD at room temperature. After agitation for 7 days, the clear solution was lyophilized using LABCONCO (Model: Freezone 4.5). The dried powder was passed through a 100# sieve and then stored in a desiccator.

Milling method

The ball-milling process was used for preparation of the drug-cyclodextrin binary system. A ball mill of 1.25-L capacity with a height of 16 cm and a diameter of 14.4 cm (external)/11.1 cm (internal) was used. The ceramic pebbles (balls) used had average diameters of 2.2 ± 0.13 cm and average weights of 15.728 ± 0.49 g. The required 1:1 M quantity of LPV was milled for 6 h with γ-CD at room temperature. The milled powder was passed through a 100# sieve.

Autoclaving method

In the autoclaving method, the aqueous solution of γ-CD was added to the aqueous suspension of LPV to obtain a complex of 1:1 stoichiometry in conical flask. The mouth of the conical flask was closely tight with the help of cotton plug. The resulting mixture was autoclaved for 30 min at 121 °C at 15 psig. After releasing pressure, the mixture was dried under vacuum at 60 °C. The dried mass was pulverized and passed through a 100-mesh sieve.

Characterization of complex in solid state

Total four samples i.e. Plain LPV, γ-CD, physical mixture of LPV and γ-CD, complex of LPV/γ-CD were analyzed. The complexes were characterized using following analytical techniques:

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (Perkin Elmer, Pyris 6, Germany) measurements were performed using 4.0–6.0 mg accurately weighed into non-hermetically sealed aluminum pans. Samples were heated at a rate of 10 °C min^{-1} in a 30–150 °C temperature range under a nitrogen steam.

X-ray Powder Diffractometry (XRPD)

The X-ray diffractograms were obtained using on X-Ray diffraction instrument (Philips Analytical-X'Pert PRO) with Ni-filtered Cu radiation, at a voltage of 40 kV and current of 30 mA. The scanning speed was 2 degree/min between 2 and 50 theta.

Scanning Electron Microscopy (SEM) studies

Scanning electron microscopy [21] was performed to elucidate the crystal structure of test samples. The samples were mounted on metal stubs with a double-sided adhesive band and then sputtered with a 100 Å thick layer of gold. They were examined with a JEOL scanning electron microscope (JSM-6380 LA) at an acceleration voltage of 80 kV.

Pharmacokinetic studies

Pharmacokinetic studies were performed for comparative analysis of pharmacokinetic parameters after oral administration of optimized and reference tablet formulation in slurry form. A HPLC method developed for analysis of samples for assay and dissolution studies was used with slight modifications. Ambroxol was used as internal standard and the injection volume was increased from 20 to 200 μL.

The protocol for the pharmacokinetic study was approved by the CPCSEA (Protocol No. UICT/PH/IAEC/0109/24). Sprague-Dawley male rats of average body weight 200–250 g were obtained from Haffkine Institute (Mumbai, IN) and maintained according to CPCSEA guidelines. The rats were housed in polypropylene cages before and after dosing and were provided with a certified rodent pellet diet and pure drinking water. Animals were maintained under controlled conditions of temperature (22 ± 2 °C), relative humidity (55 ± 5%) and approximately 12 h light and dark cycle. The rats were acclimated for a minimum of 4 days.

Seven rats were used for the study (three for optimized tablet formulation, three for reference tablet and one control for 1% w/v gum tragacanth gel). The tablets were powdered and pass through 100# sieve. Slurry of tablet powders was prepared using mortar and pastel in 1% w/v gum tragacanth gel in water. Using the Clark's rule for conversion of dose on basis of body weight, dose for rats were calculated considering normal human dose of LPV, 200 mg and RTV, 50 mg. The rats were kept for fasting for 12 h before administration. The slurry equivalent to one dose was administered orally to fasted rats. Blood samples (approximately 1 mL) was collected from each rat via a cannula placed in the jugular vein in 1.5 mL capacity eppendorf tube containing 0.1 mL of 4% w/v dipotassium EDTA solution before oral administration (time 0) and at 1, 2, 4, 5, 5.5, 6, 6.5, 8, 12, 18 and 24 h after oral administration. Cross over studies were performed after wash out period of 3 days (more than 6 half life of LPV/r, minimum requirement is at least 3 half life). Reported half of LPV is 5–9 h and RTV is 4–6 h [22].

Plasma was separated from the blood sample by centrifugation at 3000 rpm for 15 min and stored in separate vials at 4 °C until analysis. Plasma samples were removed from the freezer and allowed to thaw at room temperature (~25 °C). Once thawed, 0.5 mL aliquot was transferred to a 10 mL centrifuge tube, to this 100 μL of the internal standard solution (100 ng/mL) was added. The resulting solution was mixed and then 1 mL of 1 M NaOH was added. The alkalinized plasma was then vortex-mixed for 10 s and 10 mL of N-hexane and diethyl ether (1:1 v/v), in two halves of 5 mL each for complete extraction was added. After addition of organic phase, the tube was vortex-mixed for 10 min with intermittently stopping to avoid gel formation. The upper organic layer was carefully transferred into another conical glass vial and evaporated to dryness at 60 °C under a gentle stream of nitrogen. The dry residue was then reconstituted with 1000 μL mobile phase and vortex-mixed for 10 s; 200 μL solution was injected for HPLC analysis.

The pharmacokinetic parameters such as area under the curve from time 0 to 24 h (AUC_{0-24}), maximum serum

concentration (C_{max}), time for maximum serum concentration (T_{max}), the apparent first order terminal elimination rate constant (K_{el}), apparent first order terminal elimination half-life ($T_{1/2}$) and mean residence time (MRT), were calculated using non-compartmental method.

Results and discussion

Significance of phase solubility studies

Phase solubility studies of LPV were performed as per classical method [17] with various CDs' used in study. The intrinsic solubility value of LPV was found 3.61 μM (equal to 2.27 μg/mL) at 25 °C in aqueous solution. Theoretically, the apparent stability constant $K_{1:1}$ (S_0) calculated on basis of intrinsic solubility (S_0) should be equal to the value of stability constant, $K_{1:1}$ (S_{int}) calculated on basis of intercept (S_{int}), but practically both found to be different, Table 1. This error may be due to low solubility of LPV ($S_0 < 1$ mg/mL) in aqueous solution [18].

Phase solubility diagram is shown in Fig. 1. It clearly indicates interactions between LPV and CDs' follow A_{L-} type phase solubility profile. The slope value was found less than unity which indicates that stoichiometry of LPV/CD complexes is 1:1 [18].

Significance of complexation efficiency

We found the CE of CDs' with LPV and the results are shown in Table 1. The CE of CDs' with LPV were found to be very low. For example; CE of β-CD and γ-CD was found to be 0.0078 and 0.0010, respectively, which indicates that 1 out of 128 β-CD molecules and 1 out of 1000 molecules of γ-CD forms a complex with 1 molecule of LPV respectively. The low value of CE suggests that the molecular interactions between CDs' and LPV are very weak and probably the complexation is due to non-inclusion phenomena.

Saturation solubility studies

Saturated solubilities of LPV in presence of CDs' were found to be directly proportional to the respective stability constant value obtained from phase solubility studies, Table 1. An exception with M-β-CD was observed, where solubility of LPV was first increased and then decreased on time. This may be due to slow equilibration between free and complexed form of LPV with M-β-CD.

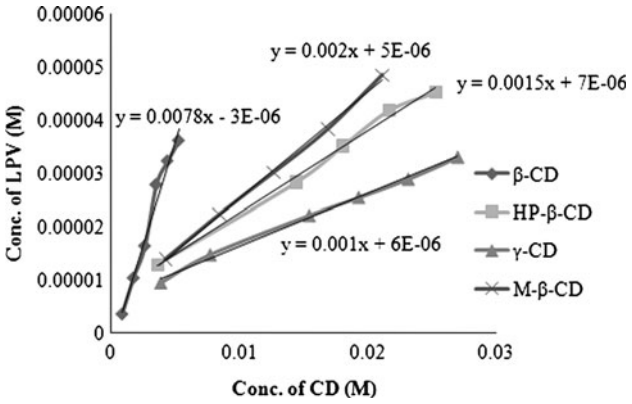

Fig. 1 Phase solubility diagram between LPV and various different cyclodextrins

In vitro release and permeation studies

Selection of cyclodextrin was performed on the basis of enhancement of rate of dissolution of LPV during in vitro dissolution studies ($N = 6$), Fig. 2. The studies were performed using 900 mL of 0.06 M polyoxyethylene 10 lauryl ether (POLE) as dissolution medium at 75 rpm with recommended sampling points of 15, 30, 60, 90 and 120 min (www.accessdata.fda.gov/scripts/cder/dissolution). The in vitro dissolution rate was compared on the basis of Q_{120} value (i.e. percentage of drug dissolved in 120 min) and it was found that LPV/γ-CD (1:1 M) complex provides maximum dissolution rate, Table 2. LPV/HP-β-CD complex (1:1 M) was found to give similar Q_{120} value as LPV/γ-CD but acquire high standard deviation. LPV/M-β-CD complex (1:1 M) possessed high dissolution rate during first 60 min., but solubility of LPV in medium decreases afterwards, this may due to slow equilibration between inter-conversion of free and complexed form of LPV. The LPV/β-CD complex results in negligible enhancement in rate of dissolution of LPV.

Table 1 Relationship between stability constant, complexation efficiency, saturation solubility and percentage dissolution

Cyclodextrin	Slope	Intercept (M)	Corr.	$K_{1:1}$ (S_0)	$K_{1:1}$ (S_{int})	CE	Saturation solubility (μg/mL)	Q_{120}
β-CD	0.0078	−3E−06	0.978	1952.72	–	0.0078	50.93	10.3
HP-β-CD	0.0015	7E−06	0.994	443.92	214.60	0.0015	49.12	51.3
M-β-CD	0.0020	5E−06	0.998	582.94	400.80	0.0020	31.88	30.3
γ-CD	0.0010	6E−06	0.996	305.04	166.83	0.0010	39.53	56.3

S_0 equals to 3.61E−06 M at 25 °C in water

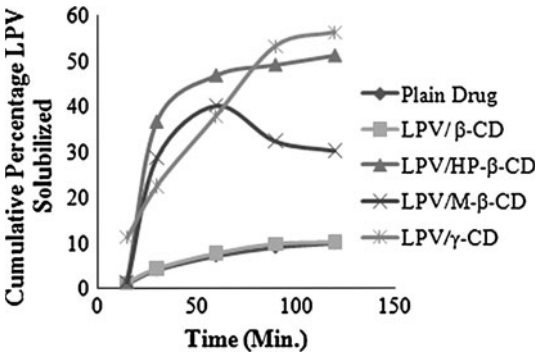

Fig. 2 Comparative in vitro release results of various LPV/CD complexes formulated by kneading method

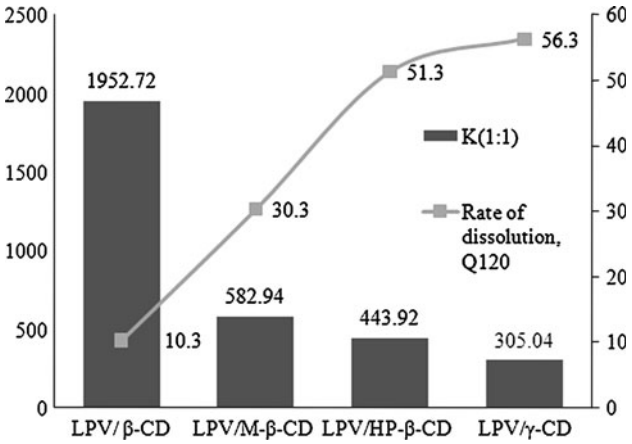

Fig. 3 Diagram showing relationship between stability constant, $K_{(1:1)}$ and rate of dissolution, Q_{120}

Anomalous results were observed, Table 1, where we found that β-CD having comparatively high stability constant value provided maximum saturated solubility with poor dissolution rate whereas γ-CD having lowest stability constant value provided lower saturation solubility with highest dissolution rate. This clearly shows a relationship, that cyclodextrin having higher stability constant value with LPV produces higher saturated solubility of LPV but at lower dissolution rate and vice versa, Figs. 3 and 4.

The effect of different cyclodextrins on flux of LPV from intestinal wall was calculated and shown in Fig. 5. It was seen that cyclodextrins enhanced the flux between 2.8 and 4 folds in comparison with the plain drug. The order for enhancement of flux was found as; M-β-CD > HP-β-CD > β-CD > γ-CD. The permeation result shows that beta-cyclodextrin and its derivatives enhanced the flux of lopinavir more than gamma cyclodextrin. There was no co-relation found between stability constant and flux. We found that the solubility of LPV follows parabolic curve with LPV/M-β-CD complex in solution i.e. solubility increases first and then start decreasing on time therefore based on combined results of in vitro release and permeation studies, we selected γ-CD for further studies.

Characterization of LPV/γ-CD complex in the solid state

The DSC thermographs of LPV from 30 to 150 °C exhibit a sharp endothermic peak at 85–95 °C, corresponding to its melting point, Fig. 6. The DSC data for γ-CD shows an endothermic peak at 95–105 °C corresponding to its dehydration. However, for the complex and the physical mixture, where the included DSC studies were performed, the above two peaks were replaced by a weak broad endotherm, spreading between 90 and 120 °C. A further endotherm was seen after the melt of LPV which must be indicative of an interaction formed in the mixture at these elevated temperatures.

According to the X-ray diffraction patterns, the inclusion complex was found less crystalline than plain LPV, γ-CD and physical mixture, Fig. 7. All the characteristic peaks of raw materials were also observed in physical as well as complex, which indicate clearly that the interactions exist between LPV and γ-CD are weak.

The SEM data shows that the complex appears to be a mere physical mixture of LPV and γ-CD, Fig. 8. All these studies indicate that there are weak interactions exist between LPV and γ-CD in complex form which can be indicative of non-inclusion complexation phenomena.

Optimization of cyclodextrin based formulation

From phase solubility studies, we found that the stoichiometric ratio between LPV and γ-CD is 1:1 on molar basis.

Table 2 Comparative rate of dissolution of various complexes

Time points	Percentage LPV solubilized					
	Plain drug	LPV/β-CD (1:1 M)	LPV/HP-β-CD (1:1 M)	LPV/M-β-CD (1:1 M)	LPV/γ-CD (1:1 M)	Reference product
Q_{15}	1.2 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	11.3 ± 0.4	44.03 ± 2.3
Q_{30}	4.2 ± 0.1	4.4 ± 0.1	36.7 ± 0.5	28.7 ± 0.3	22.6 ± 2.1	66.75 ± 3.2
Q_{60}	7.3 ± 0.2	7.8 ± 0.1	46.9 ± 2.1	40.2 ± 1.2	37.9 ± 1.3	86.08 ± 3.7
Q_{90}	9.2 ± 0.3	9.9 ± 0.2	49.2 ± 3.2	32.4 ± 1.8	53.2 ± 2.4	91.33 ± 3.5
Q_{120}	10.1 ± 0.5	10.3 ± 0.3	51.3 ± 6.3	30.3 ± 1.5	56.3 ± 3.2	97.88 ± 5.4

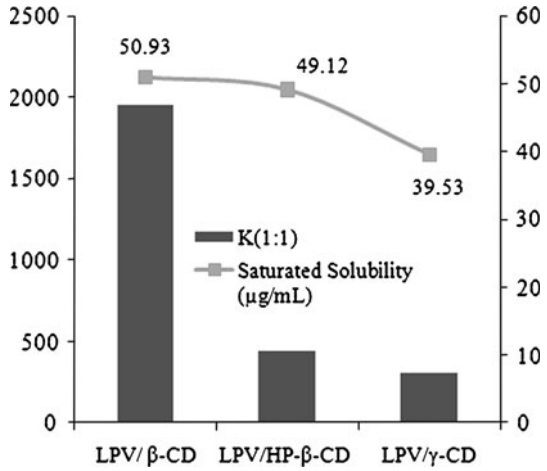

Fig. 4 Diagram showing relationship between stability constant, $K_{(1:1)}$ and saturation solubility

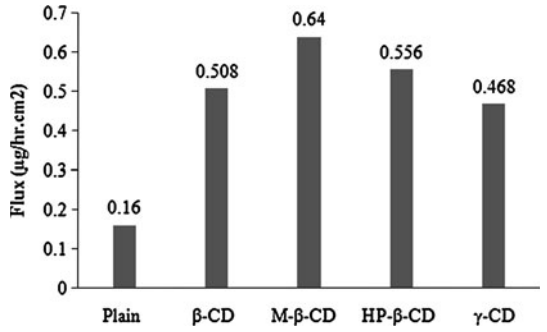

Fig. 5 Diagram showing comparative flux values of LPV obtained from various CDs' used

The LPV/γ-CD complex (1:1 M) did not enhance the dissolution rate near or equivalent to reference product based on melt extrusion technology, Table 2. To achieve more dissolution rate, we studied the effect of different ratios of LPV: γ-CD i.e. 1:1.5 M and 1:2 M. Both 1:1.5 M and 1:2 M ratio of LPV to γ-CD was found to enhance the dissolution rate more than that of reference product, Fig. 9.

To understand the cause of enhancement of dissolution rate on increase on ratio, we conducted contact angle measurement (CAM) studies. Cyclodextrin complexes are well known for reduction of surface tension between solute and aqueous solution [23]. Effect of complexation on surface tension reduction was studied using contact angle measurement (CAM) studies [24]. Contact angle (θ) measurement was performed by static sessile drop method using Kruss Contact Angle Goniometer G10. CAM studies were performed by placing a drop using syringe on compressed pellet of test substance. The pellet was compressed at pressure of approximately 4 tons using KBr pellet press. Contact angle was assessed directly by measuring the angle formed between the solid pellet and the tangent to the drop surface; Fig. 10. Direct correlation was found between reduction in contact angle and dissolution rate enhancement; Fig. 11. From these studies, it was observed that rate of dissolution of cyclodextrin complex of practically insoluble drug significantly determined by surface tension.

Various methods were tried for preparation of binary system, from which kneading method was found to be better than all other methods in terms of enhancement of rate of dissolution of LPV and reproducibility in results

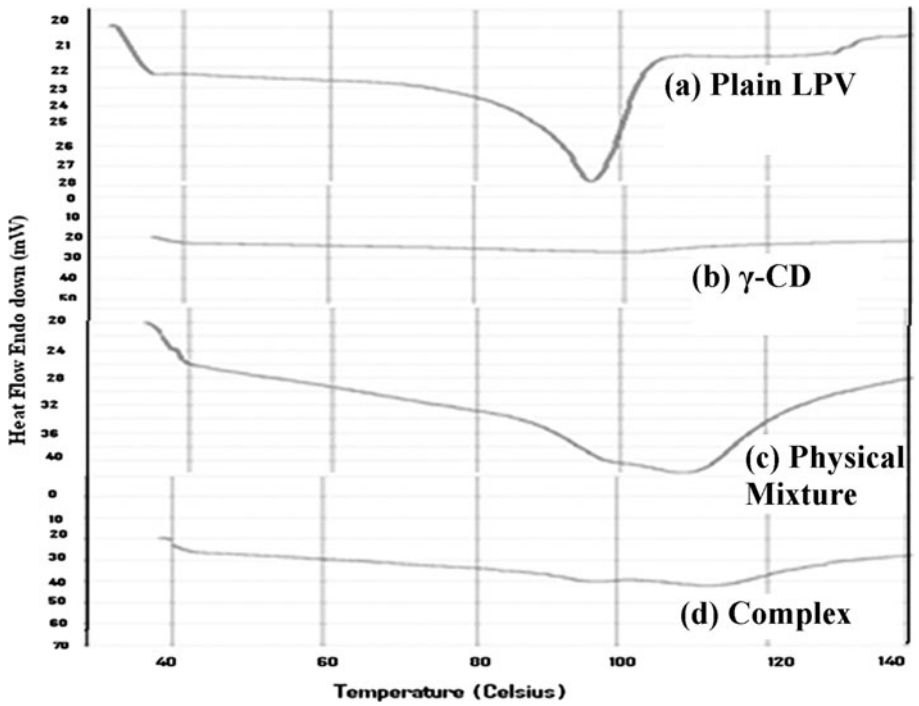

Fig. 6 DSC thermograms of (**a**) Plain LPV; (**b**) γ-CD; (**c**) Physical mixture of LPV and γ-CD (**d**) LPV/γ-CD (1:1 M) complex prepared by kneading method

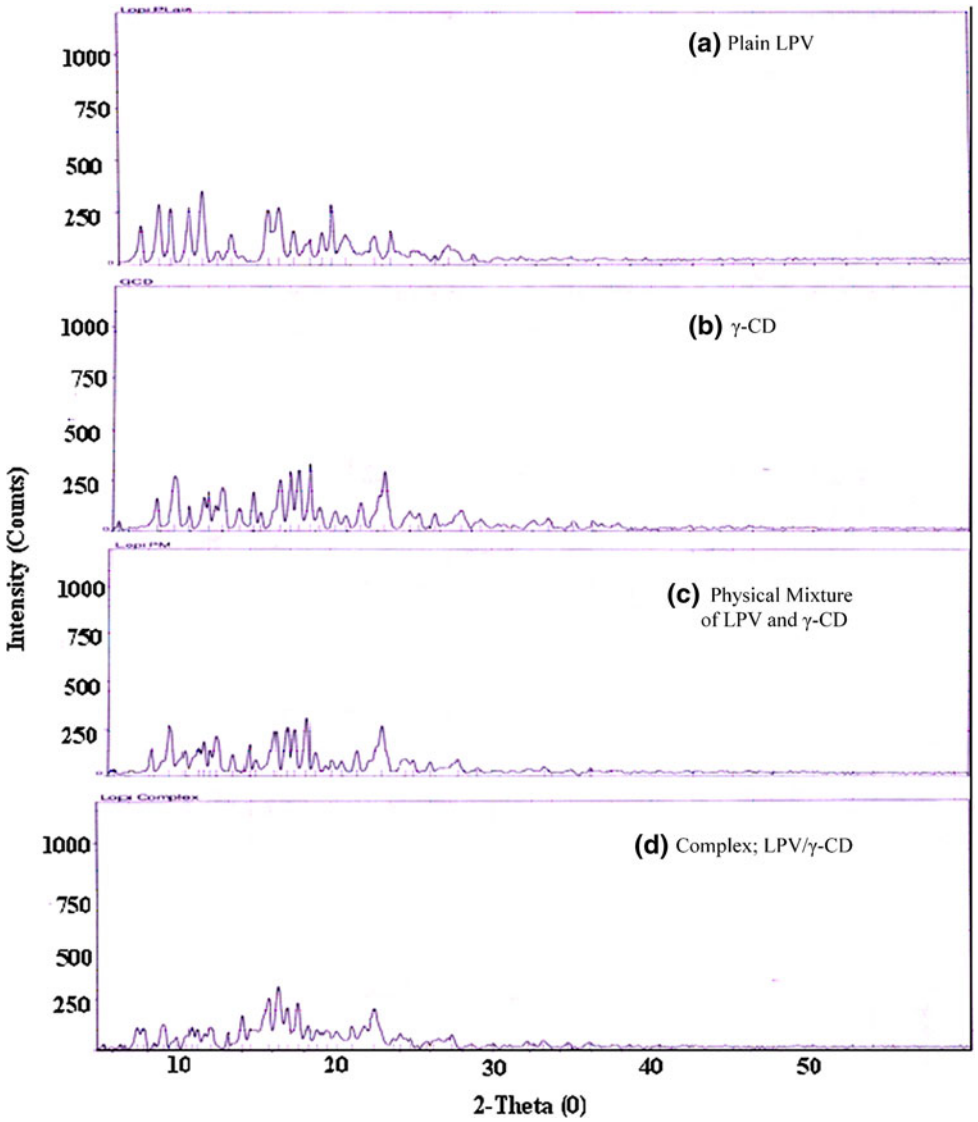

Fig. 7 X-ray diffraction patterns of (**a**) Plain LPV; (**b**) γ-CD; (**c**) Physical mixture of LPV and γ-CD; (**d**) LPV/γ-CD (1:1 M) complex prepared by kneading method

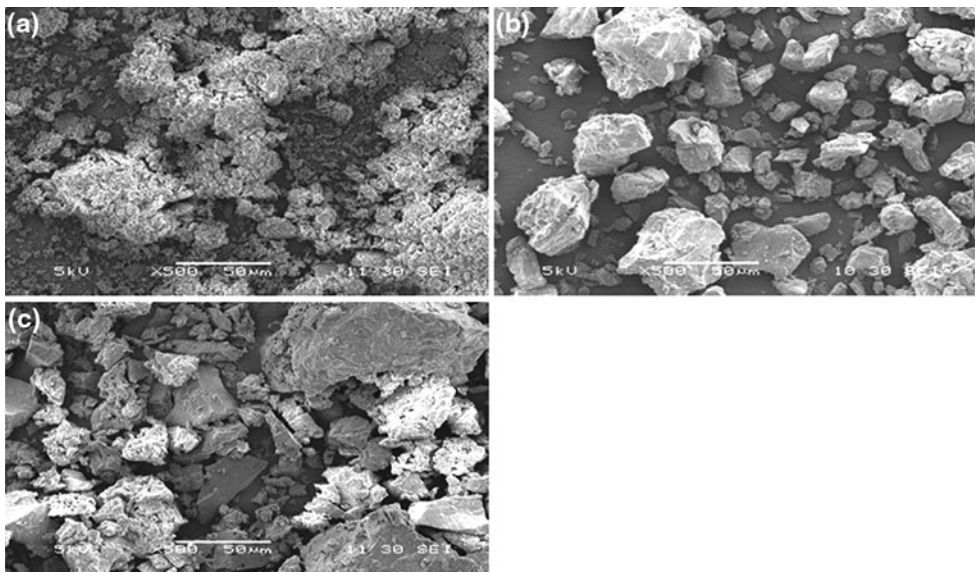

Fig. 8 SEM micrographs of (**a**) Plain LPV; (**b**) γ-CD; (**c**) LPV/γ-CD (1:1 M) complex prepared by kneading method

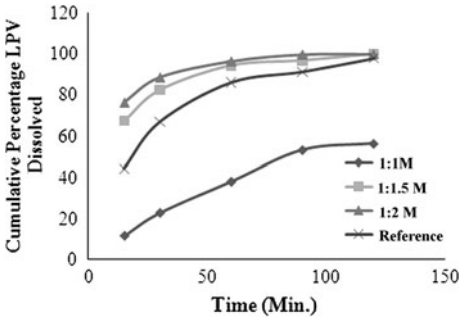

Fig. 9 Graph showing effect of different molar ratio's (1:1, 1:1.5 and 1:2 of LPV to γ-CD) on dissolution profile of LPV

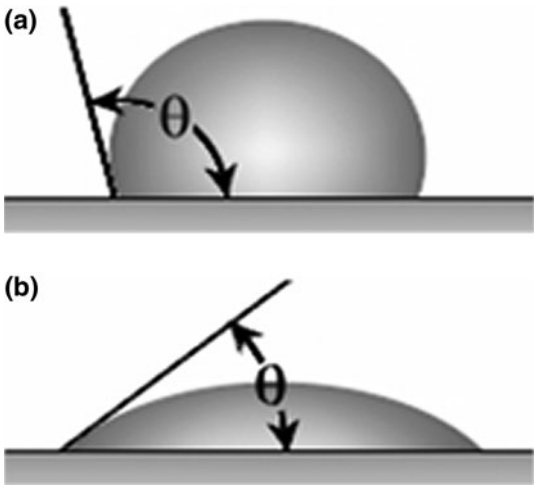

Fig. 10 Effect on contact angle (θ) with variation of surface tension. (**a**) between hydrophobic surface and aqueous drop, (**b**) between hydrophilic surface and aqueous drop

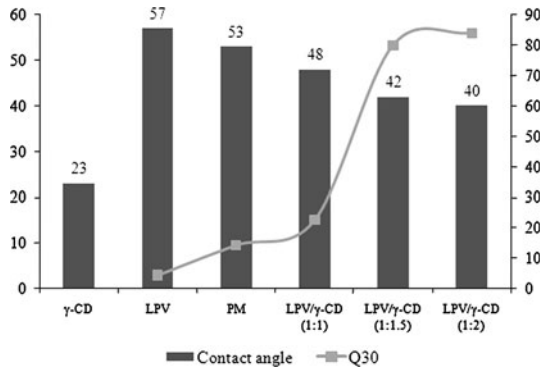

Fig. 11 Graph showing effect of different molar ratio of LPV and γ-CD on contact angle and rate of dissolution

($N = 6$), Fig. 12. Based on preliminary drug excipient compatibility studies, inactive ingredients (microcrystalline cellulose, crosscramellose sodium, magnesium stearate) were selected and processed with LPV/γ-CD (1:1.5 M) complex and plain ritonavir to get the optimized formulation.

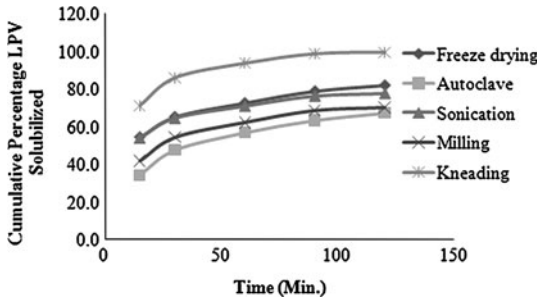

Fig. 12 Diagram showing effect of complexation technique on dissolution profile of LPV

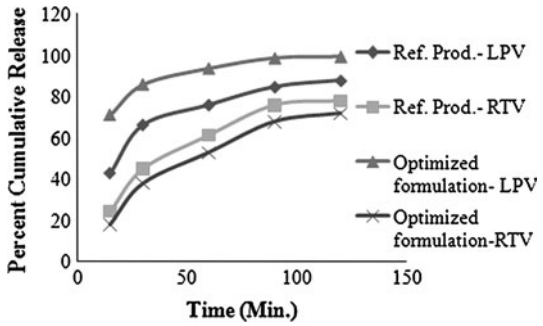

Fig. 13 Comparative in vitro dissolution profile of LPV between reference product (Ref. Product) and optimized formulation

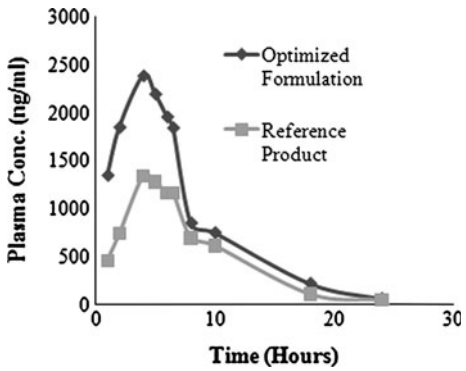

Fig. 14 Graph showing pharmacokinetic profile of LPV in fasted state obtained after oral administration of optimized and reference product

The optimized formulations was found to have less tablet weight of about 1100 mg and smaller table size (9 mm × 19 mm with 6 mm thickness) in comparison to 1400 mg of reference product with disintegration time of less than 15 min, whereas the reference product was found to be non-disintegrating. Fast disintegration can results in faster pharmacological action. The comparative in vitro dissolution profile of reference and optimized formulation is shown in Fig. 13. The similarity factor for LPV and RTV were found to be 35.9 and 56.7 calculated on basis of dissolution profile.

Table 3 Comparative pharmacokinetic parameters for LPV after oral administration of optimized and reference product in fasted condition

Hours	Optimized	Reference product
T_{max} (h)	4.1	4.08
C_{max} (ng mL)	2387.01	1337.11
AUC 0–24 (ng h/mL)	19439.6	11383.8
$T_{1/2}$ (h)	3.67	3.83
MRT (h)	7.1	7.82
K_{el} (h mL/ng)	0.19	0.18

Pharmacokinetic studies

The comparative in vivo pharmacokinetic profile obtained for LPV is shown in Fig. 14, and the pharmacokinetic calculations were performed through version 3.0 of Kinetica software and the parameters obtained are shown in Table 3. It was found that the bioavailability of lopinavir enhanced approximately 1.7 folds with optimized formulation in comparison to reference product.

Conclusions

Anomalous results were found between stability constant, dissolution rate and saturation solubility. Cyclodextrin having higher stability constant value with LPV produces higher saturated solubility of LPV but at slow dissolution rate and vice versa. From studies, we conclude that for generic product development, selection of cyclodextrin should not only be done on basis of solubility enhancement efficiency or stability constant value but dissolution profiling should also be considered. Complexation efficiency of γ-CD with LPV was found to be very small, which mainly possible due to non-inclusion complexation phenomena. Non-inclusion complexation phenomena between LPV and γ-CD was also confirmed through XRPD and SEM studies in solid state. A small, fast disintegrating, fixed dose tablet formulation of lopinavir and ritonavir with fast disintegration and higher bioavailability of lopinavir was developed.

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