



Enhancement of dissolution of Cilostazol by complexation method using Cyclodextrins

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ABSTRACT

The study was designed to investigate the effect of cyclodextrins (CDs) on the solubility, dissolution rate, and bioavailability of cilostazol by forming inclusion complexes. Natural CDs like β -CD, γ -CD, and the hydrophilic β -CD derivatives, DM- β -CD and HP- β -CD, were used to prepare inclusion complexes with cilostazol. Phase solubility study was carried out and the stability constants were calculated assuming a 1:1 stoichiometry. Solid cilostazol complexes were prepared by coprecipitation and kneading methods and compared with physical mixtures of cilostazol and cyclodextrins. Prepared inclusion complexes were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry (DSC), and X-ray diffraction (XRD) studies. *In vitro* dissolution study was performed using phosphate buffer pH 6.4, distilled water, and HCl buffer pH 1.2 as dissolution medium. The optimized inclusion complex was studied for its bioavailability in rabbit and the results were compared with those of pure cilostazol and Pletoz-50. Phase solubility study showed dramatic improvement in the solubility of drug by formation of complexes, which was further increased by pH adjustment. The *in vivo* study revealed that DM- β -CD increased the bioavailability of cilostazol with low variability in the absorption. Among all cilostazol-cyclodextrins complexes, cilostazol-DM- β -CD inclusion complex (1:3) prepared by coprecipitation method showed 1.53-fold and 4.11-fold increase in absorption along with 2.1-fold and 2.97-fold increase in dissolution rate in comparison with Pletoz-50 and pure cilostazol, respectively.

Key words:

Bioavailability,
Cilostazol-CD inclusion complex,
Dissolution, Solubility.

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INTRODUCTION

Cilostazol, {6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)-quinolinone} (Fig. 1) (1), is a cyclic adenosine monophosphate (cAMP) phosphodiesterase III inhibitor, inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. Cilostazol is slightly soluble in methanol, ethanol, and practically insoluble in water, 0.1 N HCl and 0.1 N NaOH. The reported Log *P* value is 3.048 (<http://www.chemspider.com/Search.aspx>; accessed August 15 2008). The pharmacokinetic parameters of cilostazol following oral administration are generally highly variable. Peak plasma concentration has been shown to be 1.2 μ g/mL after a single oral dose of 100 mg, generally obtained 3 to 4 h after oral administration¹.

Cilostazol absorption in the gastrointestinal tract is slow, variable, and incomplete. A highfat meal increased absorption, with approximately 90% increase in *C*_{max} and a 25% increase in area under curve (AUC) (1). The absolute bioavailability of cilostazol is not known and relative bioavailability is unpredictable. After oral administration, approximately 56% cilostazol and its metabolites (19%) are eliminated through urine (74%) and the remaining is excreted in feces (20%). The apparent elimination half-life of cilostazol and its active metabolite is 11–13 h in adults with normal renal functions². The aim of the study was to develop the inclusion complexes of cilostazol to enhance the solubility and oral bioavailability.

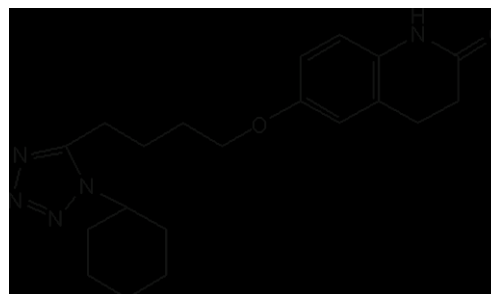


Fig 1. Chemical structure of cilostazol

Cyclodextrins (CDs) are cyclic oligosaccharide consisting of at least six α -(1-4)-linked glucopyranose units. α -, β -, and γ -CD consist of six, seven, and eight glucopyranose units, respectively (2). These are often depicted as hollow truncated cones with exterior hydrophilic surface and an electron-rich hydrophobic interior surface. Exterior hydrophilic surface is favorable for enhancement of absorption rate through the gastrointestinal tract and the hydrophobic cavity generally provides a favorable environment for hydrophobic molecules or parts of a molecule thus improving the solubility of hydrophobic compounds in aqueous solutions^{3,4}. The solubilization abilities of CDs have been attributed to the formation of inclusion complexes between CDs and the “guest” molecules. Generally, this complexation involves the inclusion of the “guest” molecule in the cavity of the host molecule, such as CD, with no covalent bonding⁵. However, practical use of natural CDs as cilostazol

carriers is restricted by their lower aqueous solubility. Several hydrophilic CD derivatives have been used to solve problem (i.e., the methylated, hydropropylated, and sulfobutyl ether CD derivatives, etc.)⁶⁻⁸. All these derivatized CDs offer better solubility, higher aqueous stability, and the increased bioavailability and have less undesirable side effects compared to natural CDs⁹⁻¹².

MATERIAL AND METHODS

MATERIALS

Cilostazol was generously provided by Cadila Pharmaceutical Laboratory, Ahmedabad, India, as a gift sample. β -CD was purchased from Hi-media Laboratories Pvt. Ltd. Mumbai. Hydroxypropyl- β -cyclodextrin (HP- β -CD) was obtained as a gift sample from Sun Pharma Advance Research Company, Vadodara, India. γ -CD and dimethyl- β -cyclodextrin (DM- β -CD) were procured as gift samples from Roquette Pharma, USA. High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from SDFine chemicals (Maharashtra, India). All other chemicals used were of analytical grade and used as received without further purification. Triple-distilled water was used throughout the study. The HCl buffer pH 1.2 and phosphate buffer pH 6.8 were prepared as per IP-1996 procedure⁽¹³⁾.

Phase Solubility Study

The analytical method based on UV spectrophotometry was developed before starting the phase solubility study. The calibration curve was prepared by measuring the absorbance of standard methanolic solutions of cilostazol in the concentration range 5–50 $\mu\text{g/mL}$ at 257.00 nm. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The coefficient of determination value (R^2) for the calibration curve was 0.9998. The intra day and inter day (3 days, $n=3$) accuracy was 99.58–100.32% and 99.80–100.4%, respectively. The intraday and interday (3 days, $n=3$) precision was expressed as relative standard deviation and were in range of 1.39–1.98% and 1.37–1.97%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were 0.231 and 0.770 $\mu\text{g/mL}$, respectively. The phase solubility of cilostazol was conducted according to Higuchi and Connors⁽¹⁴⁾. An excess amount of cilostazol (50 mg) was added to 5 mL of water or aqueous solutions of CD and its derivatives (10–50 mol/L) individually in 10 mL stoppered glass tubes. The tubes were shaken for 24 h at 50 cycle per minute in a water Bath at $37 \pm 0.5^\circ\text{C}$. At equilibrium after 2 days, aliquots were withdrawn, filtered (0.45- μm cellulose nitrate filters), and suitably diluted. Concentration of cilostazol was determined spectrophotometrically at 257.0 nm. The phase solubility study was further carried out in HCl buffer pH 1.2⁽¹³⁾ and phosphate buffer pH 6.8⁽¹³⁾. A plot of total molar concentration of the cilostazol against the total molar concentration of CDs gave phase solubility diagrams from where the apparent solubility constant, K_c , was calculated for all the pH values using their regression lines to the following equation.

Stability constant (K_c) = $\text{Slope} \times S_0 / (1 - \text{Slope})$

Where S_0 is the intrinsic solubility of the cilostazol studied under the conditions.

K values

β -cyclodextrin = 266 M^{-1}

HP- β -cyclodextrin = 160 M^{-1}

PREPARATION OF DISSOLUTION MEDIUM

DISSOLUTION MEDIUM

0.30% sodium lauryl sulfate in water; 900 mL.

Apparatus 2 : 75 rpm.

Time : 60 minutes.

DETERMINE THE AMOUNT OF $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ DISSOLVED USING THE FOLLOWING METHOD

Standard solution

10 mg of cilostazol was taken in to a 10 mL volumetric flask, to it few mL of methanol was added to dissolve the drug completely. Then the volume was made up to the mark using distilled water.

Procedure

Determine the amount of $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ dissolved by employing UV absorption at the wavelength of about 257 nm on the test solution in comparison with the Standard solution, using a 1 cm cell and Medium as the blank. Calculate the amount of $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ dissolved, in percentage, by the formula:

$$C_S \times A_U \times 900 \times 100 / 900 (A_S \times L)$$

which C_S is the concentration, in mg per mL, of USP cilostazol sponges of cilostazol obtained from the Assay preparation and the RS in the Standard solution; A_U and A_S are the absorbances obtained from the Test solution and the Standard solution, respectively; 900 is the volume, in mL, of medium; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances

Not less than 80% (Q) of the labeled amount of $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ is dissolved in 60 minutes.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium, Apparatus 2, Standard solution, Test solution, and Procedure Proceed as directed for Test 1.

Time: 30 minutes.

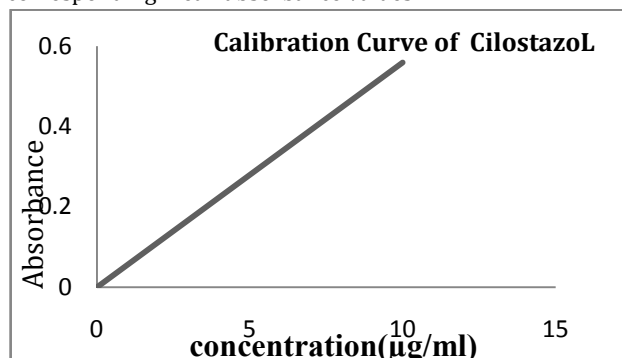
Tolerances: Not less than 75% (Q) of the labeled amount of $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$ is dissolved in 30 minutes.

Calibration curve values for the estimation of cilostazo

Concentration [$\mu\text{g/mL}$]	Absorbance*
2	0.112 \pm 0.03
4	0.224 \pm 0.02
6	0.336 \pm 0.05
8	0.448 \pm 0.1
10	0.56 \pm 0.03

*Values indicate Mean \pm S.D ($n=3$)

The standard solution was subsequently diluted with distilled water, to a series of dilutions of 2, 4, 6, 8, 10 μg . Then the absorbance of the prepared dilutions were observed using UVspectrophotometer at 257 nm. The calibration curve for the estimation of was constructed by plotting linear best fit between the concentration of cilostazol and the corresponding mean absorbance values.



S.NO	Formulation	Drug : polymer
1	F1	Pure drug
2	F2	1:1 (Cil : β CD)
3	F3	1:2 (Cil : β CD)
4	F4	1:3 (Cil : β CD)
5	F5	1:4 (Cil : β CD)
6	F6	1:1 (Cil : HP- β CD)
7	F7	1:2 (Cil : HP- β CD)
8	F8	1:3 (Cil : HP- β CD)
9	F9	1:4 (Cil : HP- β CD)

Different formulations of drug and polymer

PREPARATION OF INCLUSION COMPLEXES

The CDs used for the preparation of inclusion complexes were β -CD and HP- β -CD. Cilostazol-CDs inclusion complexes were prepared in 1:1, molar ratio by using two different methods: (1) solvent method and compared with physical mixtures of cilostazol-CDs.

(a) Physical mixture

The physical mixtures were prepared by mixing pulverized powder of cilostazol with β -CD and HP β -CD mixture was prepared.

(b) Solvent evaporation method

Dissolve HP - β CD and β - CD separately with Cilostazol in methanol and then evaporate the solvent under room temperature to produce a solid.

(c) Fluidised bed coating process

Deposition of HP β -CD/Cil solid dispersions on non -peril cores was performed in a fluid-bed coater. Firstly, Cilostazol and HP β -CD were dissolved in ethyl alcohol under continuous stirring and heating at 60 $^{\circ}\text{C}$ in a water bath till a homogenous solution was obtained. The volume of the drug/carrier solution was adjusted accordingly to achieve a final HP- β CD concentration of 10% (w/v). The solution was then sprayed through a nozzle onto the fluidized non-peril cores. The detailed operating conditions were as follows : inlet air temperature ,50 $^{\circ}\text{C}$; product temperature,32-35 $^{\circ}\text{C}$;air flow rate,97-103m 3 /h; spray rate,1.0 ml/min; atomizing pressure,1.5-1.6 bar; spray nozzle diameter,0.5mm.After

drug/carrier layering, the pellets were dried for a further 15 min at 35 $^{\circ}\text{C}$ in a coating chamber. Batch size, calculated on the basis of non-peril cores, was 50 g. Pellets were gathered and stored in sealed containers until analysis.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The IR spectra of cilostazol, β -CD and HP β -CD were studied. All the principle absorption bands were observed in the spectrum of the complexes also. This indicated that there is no incompatibility among the drug and the excipients. Pure cilostazol was characterized by aromatic and aliphatic C-H stretching peaks at 2,867 to 3,315 cm^{-1} , C=N stretching of tetrazole at 1,757 cm^{-1} , N-H stretching of quinolinone at 3,315 cm^{-1} , N=N stretching of tetrazole at 1,687 cm^{-1} , aliphatic C=O stretching band at 1,822.61 cm^{-1} , and aromatic C=C stretching band at 1,506 cm^{-1} . The FTIR spectra of β -CD, HP- β -CD, and showed intense bands at 3,465-3,247 cm^{-1} corresponding to absorption by hydrogen-bonded OH groups. The bonds that appeared at 3,000-2,800 cm^{-1} were assigned to stretching vibration of the bonds in -CH and -CH $_2$ groups. In the physical mixtures of cilostazol-CDs, the spectra were the superimpositions of those of the pure compounds with attenuation of the cilostazol peaks. However, the spectra of inclusion compounds showed rightward shifts of the band corresponding to hydrogen-bonded OH groups (from 3,352.05 to 3,315 cm^{-1} for the cilostazol- β -CD complex, from 3,380.98 to 3,313 cm^{-1} for the cilostazol-HP- β -CD complex). These results suggested that some of the existing bond formed between the OH groups on the narrow side of CD molecules might be disturbed after the formation of inclusion complexes.

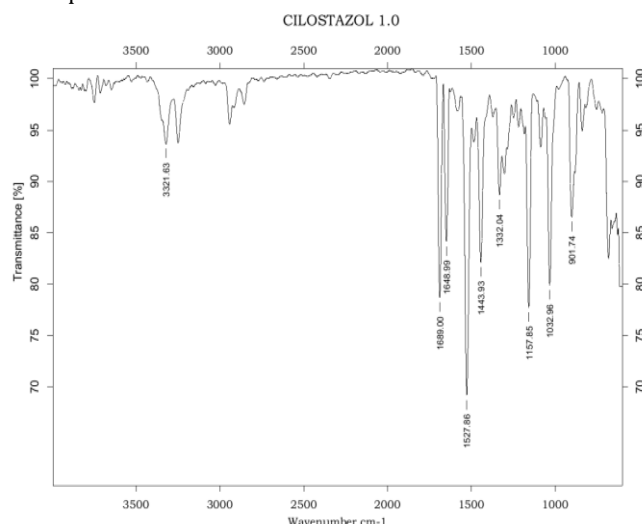


Fig 2: FTIR spectrum for the cilostazole pure sample



Fig 3: FTIR spectrum for the cilostazole and cyclodextrin mixture

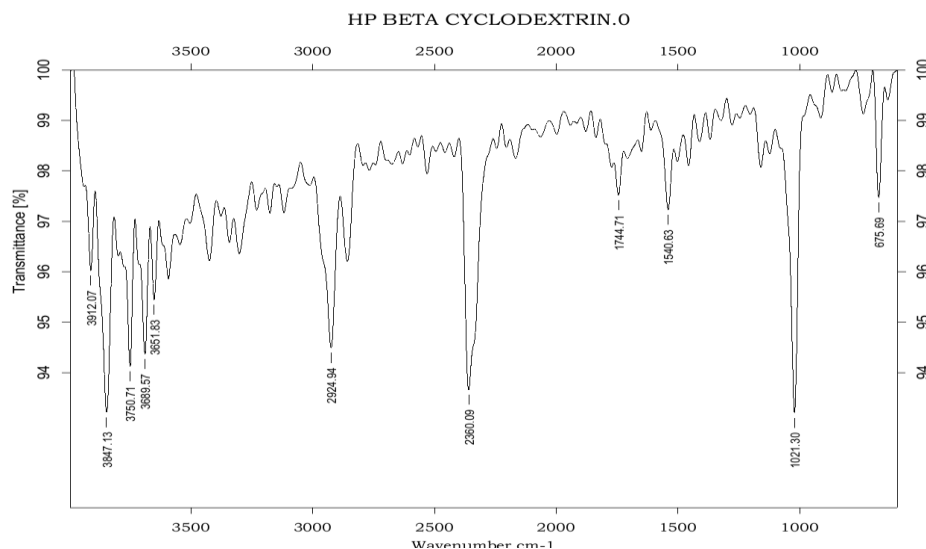


Figure 4 : FTIR spectrum for the cyclodextrin

ASSAY OF PREPARED COMPLEX

In this study ,cilostazol in solid dispersion pellets and dissolution media was determined by an UV method.

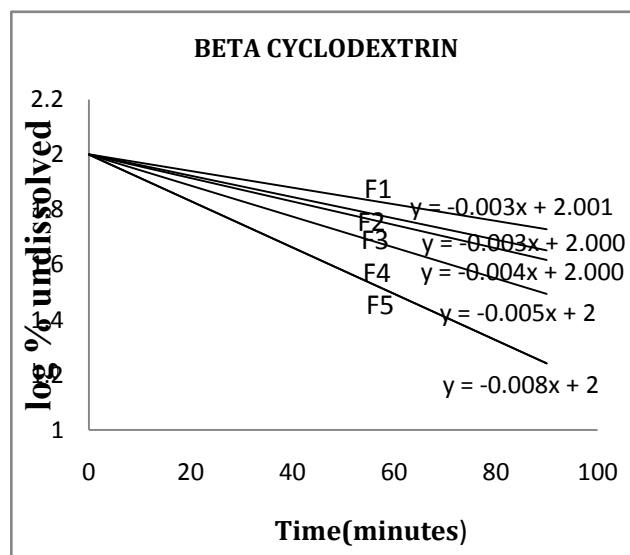
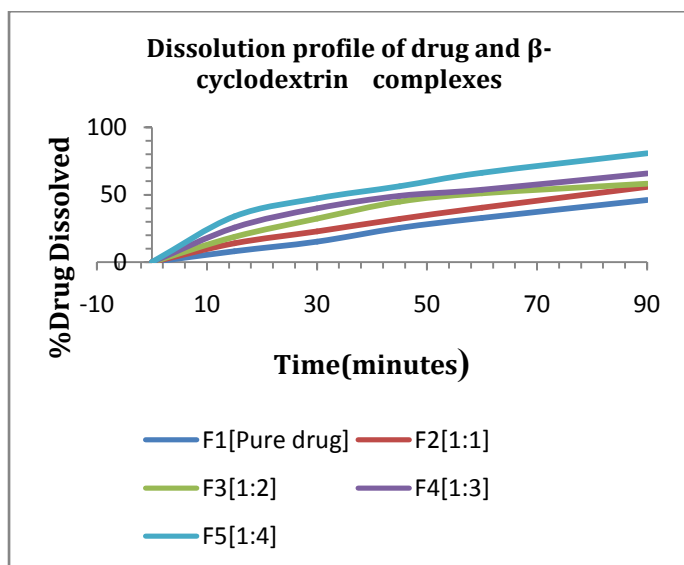
DISSOLUTION STUDIES

The dissolution studies were conducted using a LABINDIA dissolution tester based on Pharmacopoeia Method II [Paddle method] .samples containing 100mg Of cilostazol was placed n bowl and mmerged n 900ml iiof ii0.3% SLS solution thermostatically maintained at $37 \pm 0.5^\circ\text{C}$ at a rotation rate of 75 rpm at appropriate time intervals,5ml of the sample was withdrawn and replaced in order to maintain sink conditions and filtered. The filtrate was analysed by UV for estimation of cilostazol.

Dissolution rate test profile of drug and β -cyclodextrin complexes

S. No	Time	%Drug dissolved*				
		Pure drug	F1 [1:1]	F2 [1:2]	F3 [1:3]	F4 [1:4]
1	15	8.1 \pm 0.01	13.9 \pm 0.02	18.8 \pm 0.6	25.6 \pm 0.06	33.9 \pm 0.04
2	30	15.2 \pm 0.05	22.8 \pm 0.08	32.3 \pm 0.05	39.8 \pm 0.03	47.3 \pm 0.09
3	45	25.3 \pm 0.07	31.9 \pm 0.03	44.8 \pm 0.08	49.1 \pm 0.05	56.2 \pm 0.05
4	60	32.8 \pm 0.04	40.2 \pm 0.02	50.9 \pm 0.12	53.6 \pm 0.02	68.9 \pm 0.03
5	90	45.9 \pm 0.1	55.8 \pm 0.07	58.1 \pm 0.01	65.7 \pm 0.09	80.6 \pm 0.02

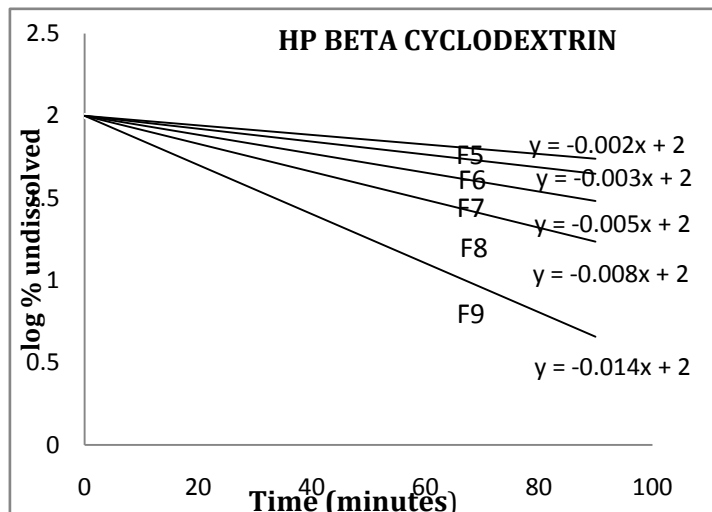
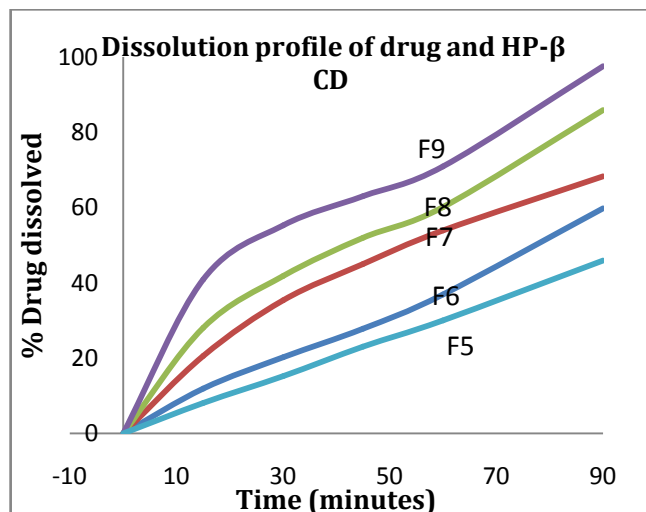
*Values indicate Mean \pm SD (n=6)



Dissolution rate test profile of drug and HP-β cyclodextrin

S.NO	Time	%Drug dissolved*				
		Pure drug F5	F6 [1:1]	F7 [1:2]	F8 [1:3]	F9 [1:4]
1	15	8.1±0.01	11.9±0.04	20.6±0.02	28±0.03	40.9±0.03
2	30	15.2±0.05	20.2±0.02	35.4±0.09	41.8±0.08	55.3±0.05
3	45	25.3±0.07	27.8±0.07	47.2±0.04	51.6±0.01	63.9±0.08
4	60	32.8±0.04	36.9±0.03	53.9±0.03	60.1±0.03	70.9±0.01
5	90	45.9±0.1	59.8±0.12	68.3±0.05	85.9±0.05	97.6±0.13

*Values indicates that Mean ± SD (n =6)



CONCLUSION

Complexes have been successfully prepared by deposition of the co-precipitates of Cilostazol /HP-β CD done on non peril pellets using a fluid –bed coating technique. The dissolution of the HP-β CD/Cil solid dispersions was enhanced greatly at HP-β CD /Cil ratios of over 4/1 and a coating weight gain of about 100%. It is indicated that the fluid bed coating technique can possibly be used to deposit solid dispersions on non-perils and may find application in the manufacturing and scaling-up of solid dispersion formulations in the future. So that F9 formulation shows enhanced solubility, in order to increase the bioavailability of cilostazol.

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