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FORMULATION AND EVALUATION OF CYCLODEXTRINS BASED CARVEDILOL SOLID INCLUSION COMPLEXES BY LYOPHILIZATION METHOD

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ABSTRACT

Cyclodextrins are cyclic (α-1, 4)-linked oligosaccharides β-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface, which have been extensively used to increase aqueous solubility of carvedilol (CVD). The phase solubility of the drug indicated the formation of 1:1 Molar and 1:2 Molar inclusion complexes in solution with β-cyclodextrin (β-CD) and 2-hydroxy-propyl-β-cyclodextrin (HP-β-CD). The solid complexes were prepared by Lyophilization method and characterized by differential scanning calorimetry (DSC), FTIR, Scanning electron microscopy (SEM) and UV-Spectrophotometry. Complexes were analyzed for drug content and the increment in the aqueous solubility. Carvedilol was found to be 17.5 times and 34.16 times greater in β-CD-CVD complex and HP-β-CD-CVD complex (in 1:2 Molar) respectively, than pure drug alone. The solubility of HP-β-CD-CVD in 1:1 Molar was found to be 2.05 times increment than β-Cyclodextrin-drug complex and 1.952 times more in HP-β-CD-CVD complex in 1:2 Molar than β-CD-CVD complex. Thus, the cyclodextrins provided significant increment in aqueous solubility of drug molecule.

KEYWORDS: Cyclodextrins, solubilization, Inclusion complex, Carvedilol, Solubility

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INTRODUCTION

Carvedilol (CVD) is a β-adrenergic receptor antagonist with β-adrenergic receptor antagonist activity that has been approved for the treatment of essential hypertension and symptomatic heart failure. The ratio of α_1 to β-adrenergic receptor antagonist potency for Carvedilol is 1:10[1]. Cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients[2]. These are cyclic (α-1, 4)-linked oligosaccharides or β-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface, which have been extensively used to increase aqueous solubility of poorly soluble or insoluble drugs[3]. From various types of Cyclodextrins reported in the literature β-cyclodextrin (**β-**CD) and hydroxy-propyl-β-cyclodextrin (HP-β-CD) were selected for present work due to numerous advantages such as they have a well defined chemical structure that provides a number of potential sites for chemical modification or conjugation, availability of different cavity sizes, low toxicity and low pharmacological activity, good aqueous solubility and protection of the included conjugated drugs from biodegradation[4]. These have no erythema, edema, and are non-irritant and non mutagenic to skin[5]. Therefore it was proposed to develop inclusion complexes of Carvedilol using these Cyclodextrins by lyophilization method[6] for improving the solubility of carvedilol. The present study was aimed at the preparation of cyclodextrin based solid complexes and their evaluation.

MATERIALS AND METHODS

The sample of Carvedilol used in this study was generously provided by M/s Cipla Limited Mumbai, as a gift sample. β-CD and HP-β-CD were purchased from Himedia, Mumbai. All other chemicals and solvents used in this study were of analytical grade. Freshly distilled water was used throughout the work.

(i) Estimation of Carvedilol

For estimation of Carvedilol ultraviolet (UV) spectrophotoscopic method was used, which was based on measurement of absorbance at 242.0 nm in methanol[7]. The concentration was obtained from corresponding absorbance using calibration curve prepared in the concentration range 2-20 µg/ml in methanol. The absorbance can be determined by using equation developed by this method as y = 0.0519x + 0.014.

(ii) Phase Solubility Studies

Initially phase solubility studies of carvedilol in β-CD and HP-β-CD were carried out according to Higuchi and Connors[8]. In each case an excess amount of drug was added to the aqueous solution of respective cyclodextrin at varying concentrations (3-15 mM/L) in a series of 25 ml Stoppard amber colored volumetric flasks and the contents were stirred in a water-bath incubator shaker for 72 hours at room temperature (25^0). After equilibration the samples were filtered through What-man filter paper no.41 and diluted suitably and assayed for concentration of drug in solution against similar concentration of β-CD and HP-β-CD in distilled water as blank, so as to nullify any absorbance that may be exhibited by the β-CD and HP-β-CD molecules by Simadzu UV-1601 spectrophotometer. From this study optimized molar ratios for complex formation of Carvedilol in β-CD and HP-β-CD were selected for further studies. Apparent stability constant (Kc) of prepared complexes were determined by following equation- Kc= Slope/ S0 (1- Slope) .Where So is solubility of drug in absence of cyclodextrins[13,14].

(iii) Preparation of inclusion complexes

From phase solubility study it was found that the drug can complex with cyclodextrins (CDs) in the molar ratio of 1:1 and 1:2 for maximum solubilization in water. Thus solid inclusion complexes of carvedilol and

cyclodextrins (β-CD and HP-β-CD) were prepared in 1:1 and 1:2 molar ratios by lyophilization method. This method involves generation of solid dispersions of Carvedilol with cyclodextrins by freeze drying of drug cyclodextrin complex in water, which were frozen in liquid nitrogen and lyophilized (Heto Drywinner, Thermo Scientific, USA) for 48 h at -70 ˚C, at a 0.05 mm Hg pressure. For preparation of 1:1 molar complexes an equi-molar concentration of carvedilol and corresponding Cyclodextrins (6.6 mM for β-CD and HP-β-CD) were dispersed in 20 ml of distilled water separately in amber colored Stoppard volumetric flasks. These mixtures were agitated for 72 hours on water-bath incubator shaker at room temperature (25^0) and filtered through What-man filter paper, the filtrate was lyophilized and resultant solid complexes were stored in amber colored vials for further studies. For preparation of 1:2 molar complexes the molar concentration of carvedilol corresponding Cyclodextrins (13.3 mM for β-CD & HP-β-CD) were dispersed in 20 ml of distilled water in amber colored stoppered volumetric flasks following same procedure as for 1:1 molar complex preparation. Freeze dried samples were stored in amber colored vials at room temperature. The physical mixtures of carvedilol with β-CD and HP-β-CD were prepared by triturating together same amount of cyclodextrins and drug as for 1:1 and 1:2 molar complexes in a clean, dry, glass pestle mortar for 25 minutes. These were stored in amber colored vials for further studies.

(iv) Characterization of Solid Inclusion Complexes

The prepared complexes were characterized and evaluated by FTIR, DSC, UV absorption and Electron microscopy. For the FTIR analysis spectra of samples were recorded on Perkin–Elmer 16 pc FTIR instrument using the KBr disc technique. Previously dried sample (5 mg) was mixed with 85 mg of KBr in a clean glass pestle and mortar. The contents were compressed by IR pelleting machine to get the pellets. These pellets were then used for scanning in the range of 4000 to 500 cm^{-1} for recording spectrum. The thermal behavior of each inclusion complex was determined using 2910 modulated DSC, thermal analyst 2000 TA instrument, at a heating rate of 10^0/min over temperature range of 40–150^0, for sample size 2-5 mg. UV absorption spectra of carvedilol, Cyclodextrins and corresponding inclusion complexes were determined after appropriate dilutions with water and methanol for scanning on GBC Cintra-10 UV-visible spectrophotometer and Shimadzu UV-1601 Spectrophotometer (using liquid form of each sample). SEM of samples were performed using Jeol Scanning electron microscopy JSM-840 with a 10 KV accelerating voltage. The sample were put on the grid and fixed by adhesive and used for photo microscopy, without metal coating.

(v) Determination of aqueous solubility of complexes

For analysis of prepared complexes and determination of aqueous solubility of complexes the most commonly used method is to extract the guest from the complex. Amount equivalent to 10 mg of drug-β-CD and HP-β-CD-drug complex (1:1 and 1:2 molar) were added to a series of screw-capped test tubes. Ten ml water was added in each tube. The tubes were shaken and placed in a water bath at 60^0 for about one hour. The tubes were also shaken periodically during the incubation period. The mixture was cooled and dichloromethane (DCM) was added. Cyclodextrins were soluble in water whereas drug was soluble in DCM. DCM was evaporated under vacuum and volume made up with methanol for analysis of drug by spectroscopic method. On the basis of this analysis the aqueous solubility of the prepared complexes were determined.

RESULTS AND DISCUSSION

The solubility of carvedilol did not increase linearly with increasing concentration of β-CD

And HP-β-CD and thus phase solubility curves were characterized as Bs-type according to Higuchi and Connors in which a plateau region was reached in the solubility

curve. According to this graph the point A indicated the solubility of drug in water. With the addition of the Cyclodextrins (β-CD/HP-β-CD) the solubility of carvedilol had risen linearly owing to complexation. At point B, the solution is saturated with respect to the complex and to the drug itself. The complex continued to be formed and precipitated from the saturated system as more Cyclodextrins were added. At point C, all of the excess solid drug had passed into solution and had been converted to the complex although, solid drug was exhausted and the solution was no longer saturated. Some of the drug remained un-complexed in solution and it was combined further with Cyclodextrins to form higher complexes (Fig 1). Apparent solubility constant (Kc) obtained from the slope of the linear phase solubility diagrams for β-CD and HP-β-CD were found to be 416 M^{-1} and 427.5 M^{-1} respectively. These values indicated that formed complexes were stable. From this study the optimized molar ratio for complexation were 1:1 and 1:2 molar for further studies.

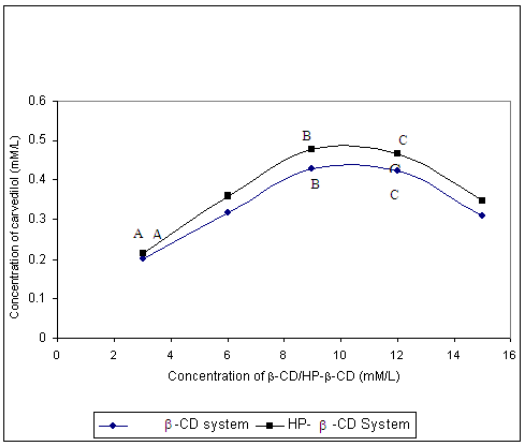

Figure 1
Phase solubility diagram of β-CD and HP-β-CD-drug complex system

The solid inclusion complexes were prepared by Lyophilization method. Pure samples and their physical mixtures were characterized for identification, confirmation and purity determination using FTIR, DSC, UV and SEM analysis methods. FTIR spectrum analysis is a highly sensitive method in which complexes showed some changes from parent spectra i.e. pure drug and cyclodextrins. Spectrum of pure carvedilol, β-CD, HP-β-CD, their physical mixtures and complexes were interpreted (Table 1, 2). The physical mixtures of β-CD-CVD system and HP-β-CD–CVD system (1:2 Molar) show most of the common peaks present in pure drug and cyclodextrins. Secondary N-H stretching peak of drug overlapped with hydroxyl bands of cyclodexrins. This indicated that minimum interaction had taken place between β-CD, HP-β-CD and carvedilol. In β-CD-CVD complex (1:1 Molar) the secondary amine N-H stretching vibration at 3346.0 cm^{-1} and C-N stretching at 1255.0 could not be detected. These indicated strong interactions between CDs and drug molecule. In β-CD carvedilol complex (1:2 Molar) similar results were found. In the secondary amine N-H stretching was completely overlapped with hydroxyl band indicating the complete complex formation between carvedilol and β-CD molecule. In HP-β-CD-CVD complex (1:1Molar) secondary N-H stretching had disappeared but C-N stretching with medium intensity was present that showed partial complex formation. In HP-B-CD-CVD complex (1:2Molar) complete absence of N-H stretching (3346.0 cm^{-1}) and C-N stretching (1255.7cm^{-1}) were found that suggested complete complexation. These observations were found to be similar to that reported in literature.[9, 10]

Table 1
IR interpretation of carvedilol β-CD-complex

Peaks at (cm^{-1})	CVD	β-CD	PM-β-CD-CVD	β-CD-CVD-Comp. (1:2M)	β-CD-CVD-Comp. (1:1M)
3400-3370.1	-	O—H str Vibrations (B, S)	O—H str. (B,W)	O—H str. (B, S)	O—H str. (B,S)
3346.6 – 3346.0	2^0N-H. str (S.S)	-	2^0NH-str (S,S)	-	-
2995.4 – 2924.2	C-H str due to methyl group (aliphatic) (S, M)	C-H str. (aliphatic S,M)	C-H str. aliphatic (S,M)	C-H str. aliphatic (S,M)	C—H str. aliphatic (S,M)
2000-1600	Overtones and combination bands				
1596.2 – 1592.8	C=C Ring str (S, S)	C=C ring str (S, S)	C=C ring str (S, S)	C=C Ring str (S, S)	C=C ring str. (S,M)
1452.1 – 1451.2	C—C ring str bending (S,S)	-	C—C ring str bending (S,S)	-	-
1347.9 – 1345.6	C—H bending methyl (S,M)	C—H methyl bending (S,M)	C—H methyl bending (S,M)	C—H methyl bending (S,M)	C—H methyl bending (S,M)
1256.0 – 1255.8	C—N str (S, S)	-	C—N str (S, M)	-	-
1217.3	O—C str (S, M)	-	O—C str. (S,M)	-	-
1157.5 – 1156.6	-	C—O str. (S,M)	C—O str. (S,M)	C—O str. (S,M)	C—O str. (S,M)
1099.0	C—O str (20 alc.) (S, S)	-	-	-	-
1033.5 – 1022.2	C—OH str (S,W)	C—OH str (equitorial) (S,W)	C—O str. (S,M)	C—O str. (S,S)	C—OH str. (S,S)
858.4 – 852.1	C—H out of plane bending (S,M)	C—H out of plane bending (S,M)	C—H out of plane bending (S,M)	C—H out of plane bending (S,M)	C—H out of plane bending (B,M)
722.9	Methylene rocking bending (S,M)	-	Methylene rocking bending (S,M)	-	-

CVD = Carvedilol; S, S = Sharp, Strong; B, S = Broad, Strong; S, M = Sharp, Medium
B, W = Broad, Weak; S, W = Sharp, Weak; Str. = Stretching
β-CD = β-cyclodextrin, PM-β-CD-CVD = Physical mixture of β-cyclodextrin-carvedilol system
β-CD-CVD-comp. = Complex system of β-cyclodextrin-carvedilol

Table 2
IR interpretation of HP-β-CD-CVD complex

Peaks at (cm^{-1})	Carvedilol	HP-β-CD	PM-HP-β-CD-CVD	HP-β-CD-CVD-Comp. (1:2M)	HP-β-CD-CVD-Comp. (1:1M)
3372.0 – 3428.7	-	O—H str vibrations (B,S)	O—H str. (B,W)	O—H str. (B, S)	O—H str. (B,S)
3346.2-3346.0	-	_		2^0 N-H str. (S,M)	
2995 – 2927.1	C-H str. aliphatic (S,W)	C—H str aliphatic (S,M)	C—H str. aliphatic (S,M)	C—H str. aliphatic (S,W)	C—H str. aliphthic (S,M)
2000 – 1600	Overtones and Combination bands				
1597.3 – 1592.8	C=C ring str. (S,S)	C=C ring str. (S,S)	C=C ring str. (S,S)	C = C ring str. (S,S)	C=C ring str. (S,S)
1504 – 1503.9	C—C ring str. (S,S)	-	C—C ring str. (S,S)	-	-
1350.3 –	C—H methyl	C—H methyl	C—H	C—H methyl	C—H methyl

1338.6	bending (S,M)	bending (S,W)	methyl bending (S,M)	bending (B,W)	bending (S,M)
1255.8 – 1255.7	C—N str. (S,S)	-	C—N str. (S,M)	-	C—N str. (S,M)
1216.8 – 1216.3	O—C str. (S,S)	-	O—C str. (S,M)	-	-
1157.9 – 1157.3	-	O—C str. (S,S)	C—O str. (S, M)	-	O—C str. (S.M)
1099.0	C—O str. (2^0alc.)(S,S)	-	-	-	-
1035 – 1022.2	C—OH str. (equitorial) (S,W)	C—OH str. (equitorial) (S,S)	C—OH str. (equitorial) (S,M)	C—OH str (B,W)	C—OH str. (B,M)
722.9 – 721.1	Methylene rocking bending (S,M)	-	Methylene rocking bending (S,M)	-	Methylene rocking bending (S,M)

CVD = Carvedilol; S, S = Sharp, Strong; B, S = Broad, Strong; S, M = Sharp, Medium
B, W = Broad, Weak; S, W = Sharp, Weak; Str. = Streching
HP-β-CD = Hydroxy propyl-β-cyclodextrin, PM-HP-β-CD-CVD = Physical mixture of hydroxylpropyl-β-cyclodextrin-carvedilol system
HP-β-CD-CVD-comp. = Complex system of hydroxy propyl-β-cyclodextrin-carvedilol

Thermal behavior of CVD-β-CD complexes and HP-β-CD complexes were studied using DSC to confirm the formation of the solid complexes (Fig 2). The carvedilol thermogram exhibited an endothermic peak at 119^0 corresponding to its melting point. The β-CD and HP-β-CD pure samples showed broad endothermic peaks at 102^0 and 70^0, respectively. The physical mixture of β-CD and HP-β-CD with carvedilol in 1:2 molar had shown the thermograph containing two peaks, one for drug and another for cyclodextrins. In β-CD-CVD physical mixture, β-CD had shown a peak at 97^0 and drug at 119^0. In this case a little interaction was found between drug and β-CD due to slight shift in β-CD peak, than pure form. In HP-β-CD-CVD physical mixture in 1:2 molar, the HP-β-CD endothermic peak had shifted at 63^0 than pure form. In complexed form of β-CD-CVD system 1:2 molar complex, a new endotherm was obtained at 77^0. These indicated the strong interactions between these molecules that suggested complete complexation. In HP-β-CD-CVD-system 1:2 molar complexes complete disappearance of the drug and HP-β-CD endotherm and development of a newer peak at 85^0, suggested strong interaction and complete complexation between these molecules. These observations were found to be similar to that reported in literature[11, 12]. The ultraviolet studies of β-CD and HP-β-CD-CVD complexes (1:1 and 1:2 Molar) suggested the disappearance of the drug peak at 242 nm that shifted to longer wavelength 245-290.0 nm indicating the complete complexation. The scanning electron micrographs of β-CD molecule showed an oval shaped structure and pure drug had crystalline nature. The features of crystals in complex form were not easily detectable. The micrograph of complex system showed an amorphous product with the presence of small size particle tending to aggregation. Thus all of above studies suggest that complexes were formed in both 1:1 and 1:2 Molar ratios of β-CD-CVD and HP-β-CD-CVD systems.

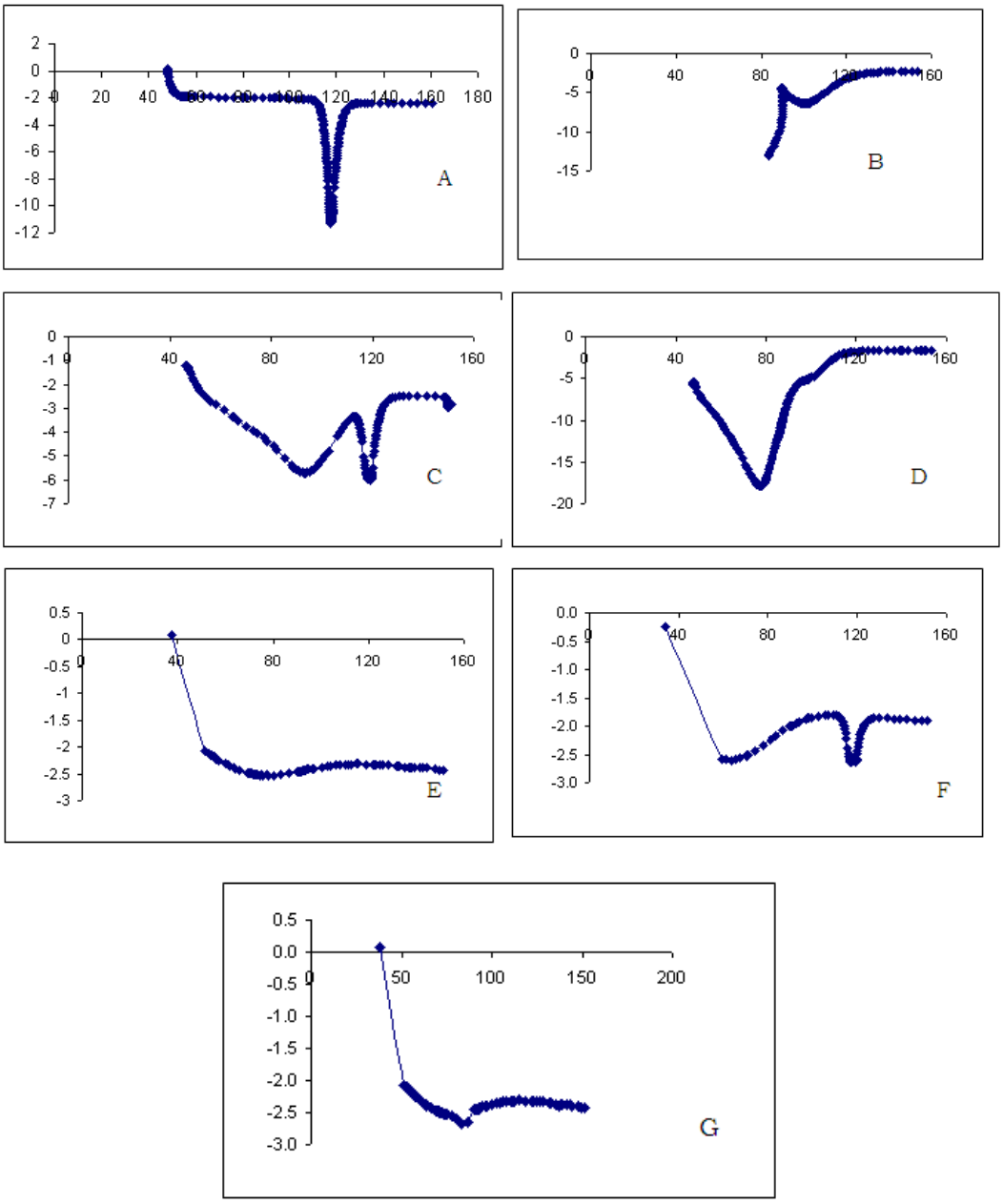

Figure 2
DSC thermograms

A = Pure carvedilol (CVD)
B = Pure β-Cyclodextrin (β-CD)
C = Physical mixture of β-CD-CVD system (1:2 Molar)
D = Pure β-CD-CVD-Complex (1:2Molar)
E = Pure HP-β-CD
F = Physical mixture of HP-β-CD-CVD system (1:2 Molar)
G = HP-β-CD-CVD complex (1:2 Molar)

Prepared solid inclusion complexes were analyzed for drug content and aqueous solubility determination. Data suggested that in β-CD-CVD complex and HP-β-CD-CVD complex of 1:2 Molar has greater drug contents and aqueous solubility than 1:1 Molar. The highest drug content and aqueous solubility were found to be in 1:2 Molar of both case (Table 3).

Table 3
Analyses of prepared inclusion complexes at 25^0

S. No.	Compounds	Water solubility * (mg/ml) (Mean ± S.D.)	No. of folds increase in carvedilol water solubility	Drug content** (mg)
1	CVD	2.4±0.042	-	-
2	β-CD-CVD-Comp. (1:1 M)	36.6±0.123	15.0	9.52 ± 0.041
3	β-CD-CVD-Comp. (1:2 M)	42.0±0.082	17.5	9.77 ± 0.062
4	HP-β-CD-CVD-Comp.(1:1M)	74.0±0.012	30.83	9.61 ± 0.056
5	HP-β-CD-CVD-Comp.(1:2M)	82.0±0.201	34.16	9.90 ± 0.034

CVD = Carvedilol, β-CD-CVD-Comp = β-cyclodextrin-carvedilol complex,
HP--β-CD-CVD-Comp = Hydroxypropyl –β-cyclodextrin-carvedilol complex
**** Results expressed as mean ± SD (n=3)***
***** Results expressed as mean±SD (n=3), the initial concentration of drug was 10 mg in each case.***

CONCLUSION

The present study clearly indicates that the water insoluble carvedilol has been successfully solubilized by using cyclodextrins and evaluated by β-CD-CVD complex and HP-β-CD-CVD complex formation by using Lyophilization method. These inclusion complexes of β-CD and HP-β-CD in 1:2 molar have greater drug content and aqueous solubility of carvedilol compared with pure drug.

CONFLICT OF INTEREST
Conflict of interest declared none.

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