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Preparation and characterization of cilnidipine hydroxypropyl-β-cyclodextrin inclusion complex by solvent evaporation to enhance oral-solubility of drug

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Abstract
The aim of this work was to improve the water-solubility of cilnidipine by complexing it with hydroxypropyl-β-cyclodextrin. Cilnidipine, a novel fourth generation calcium channel blocker, which is poorly water soluble with low bioavailability. Cilnidipine needs enhancement of dissolution rate and solubility to improve its oral bioavailability and therapeutic efficacy. The objective of this research was to prepare the inclusion complex by solvent evaporation method, evaluate the complex and to characterize it by Phase solubility method, FTIR, DSC, SEM and XRD studies. DSC and XRD analyses indicated the complete transformation of Cilnidipine in the complex from crystalline to amorphous state. The solubility of cilnidipine increased linearly as a function of HP-β-CD concentration. The results identified the cilnidipine-HP-β-CD inclusion complex as an effective new method to design a novel formulation for pharmaceutical application. It also designated that the bioavailability of cilnidipine could be improved markedly by inclusion complexation, possibly due to an increased dissolution rate.

Keywords: cilnidipine, hydroxypropyl-β-cyclodextrin, inclusion complex, solvent evaporation

1. Introduction
As most of the drugs have poor solubility in water, low bioavailability is one of the major disadvantages of oral route of drug administration. In the case of poorly water-soluble drugs, dissolution is the rate-limiting step in the process of drug absorption. When an active agent is administered orally, it must first dissolve in gastric or intestinal fluids before it can permeate the membranes of the GIT to reach systemic circulation and hence two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include. The solubility and dissolution properties of drugs play a significant role in the process of formulation development which can be solved by different technological approaches during the pharmaceutical product development work. Among the various approaches to enhance the solubility and dissolution rate of poorly soluble drugs complexation with cyclodextrin is an effective and industrially accepted technique. In recent years, inclusion complexes with cyclodextrins (CDs) have been commonly used to improve the solubility of water-insoluble drugs, enhance the physicochemical stability of drugs and improve the bioavailability [5]. Cyclodextrins (CDs) are cyclic a-1, 4 linked oligosaccharides of a-D-glucopyranose units that have relatively hydrophobic central cavity and hydrophilic outer surface [2]. They consist lipophilic inner cavity and hydrophilic outer surface, which are capable of interacting with a large variety of guest molecules by forming non-covalent inclusion complexes [6]**.** Cyclodextrins, the unique cyclic carbohydrates are successfully utilized as the potential complexing agents which form inclusion complex with insoluble drugs. β-Cyclodextrin appears to be the best natural cyclodextrin for pharmaceutical applications due to its efficient drug complexation, but the application in the pharmaceutical field is limited by its low aqueous solubility and some undesired side-effects after parenteral administration. Therefore, some chemically modified 2-Hydroxypropyl-β-cyclodextrin (HP- β -CD) have been prepared to solve such problems and improve the complexation capacity [7]. HP- β -CD has been extensively focused on, due to its excellent water-solubility and safety accord by the parenteral route. In addition, it is found that, inclusion of molecules within the cavity of HP- β -CD may protect the drug compound from the external environment, and hence, HP- β -CD may be used to optimize the chemical stability of molecules susceptible to degradation [5]. Cyclodextrin inclusion complexes can be prepared by several methods such as kneading, solvent evaporation, Spray drying and freeze-drying (Lyophilization) methods. Solvent evaporation method is quite simple and economic both on laboratory and large-scale production. This technique aiming to avoid the degradation caused by high temperature when melting method used. It is also an alternative to the spray drying technique.
Cilnidipine (CLN) [1, 4-dihydro-2, 6-dimethyl-4-(3nitrophenyl)-3, 5-pyridinedicarboxylic acid 2-methoxyethyl (2E)-3phenyl-2-propenyl ester structure depicted in figure 1] is a novel fourth generation dihydropyridine calcium channel blocker, it inhibits both L-type and N-type calcium channels in various types of neurons [6]. It has been reported to exhibit excellent clinical effects on cardiovascular diseases by inhibits cellular influx of calcium thus causing vasodilatation. Recently, CLN was found more remarkable advantages compared to traditional calcium-channel blockers, it causes a lower probability of reflex tachycardia than nisoldipine and has less influence on heart rate than nifedipine [7]. In addition, CLN also inhibits the local

renin-angiotensin system and aldosterone secretion from adrenocortical cells Thus, the pleiotropic effects of CLN on neurohumoral factors may provide a new strategy for the treatment of cardiovascular diseases, as reported in hypertensive patients with chronic renal disease [8].

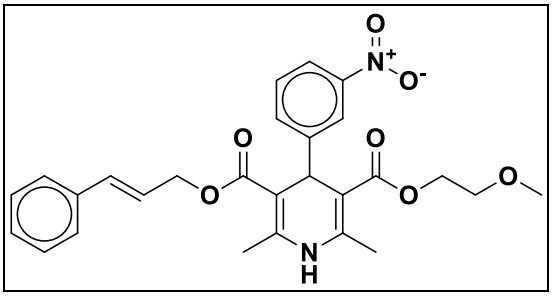

Fig 1: Chemical structure of Cilnidipine (CLN)

CLN is freely soluble in N, N-diethyl acetamide and acetone, sparingly soluble in methanol and involves in water. It is a yellow, odorless crystalline powder with molecular weight 492g/mol and it belongs to BCS class II drug. However, the dissolution and oral bioavailability of CLN are not good mainly because of its poorly water-soluble property [9].

Number of researchers have focused on the therapeutics, pharmacology and analytical methods on CLN conversely, there is limited work on developing or characterizing CLN-HP-β-CD inclusion complex. Thus, main purpose of this research was to utilize the complexation technique with HP-β-CD to improve the solubility and dissolution rate of CLN by solvent evaporation method to prepare inclusion complex of CLN with HP- β-CD and to obtain higher bioavailability of the drug. The formation of such complex was confirmed by UV/VIS spectroscopy to evaluate the absorption spectrum of complex in aqueous solution. The interactions in the solid state were characterized by DSC, PXRD analysis indicated the complete transformation of complex from crystalline to amorphous state. Finally, the dissolution rate of inclusion complex was compared with pure drug.

2. Materials and Methods

Table 1: List of materials and chemicals

S. No.	Materials	Grade	Source
1.	Cilnidipine	Analytical Grade	Ajanta Pharmaceuticals Abad & Aurobindo, Hyderabad, India
2.	2-Hydroxypropyl-β-cyclodextrin	Analytical Grade	Cyclo Lab R&D. Ltd., Budapest, Hungary
3.	Methanol, Potassium dihydrogen phosphate, Sodium hydrogen phosphate	Analytical Grade	Sigma Aldrich Pvt. Ltd., Mumbai

Phase-Solubility study of CLN

Phase solubility studies of CLN in presence of HP-β-CD was preformed according to the method disclosed by Higuchi and Connors method [10-14]. An excess amount of CLN (10mg) was added to the aqueous solution and also prepared the aqueous solution containing HP-β-CD. The concentration of HP-β-CD in solution were ranging from (1-3%) the containers were shaken at 30 rpm for 48hours at room temperature on rotatory shaker (RIME RS-12) to achieve equilibrium. After 48hours the resultant solution were filtered using 0.45µm membrane filter. The filtrate was suitably diluted and the concentration in the solution was determined by using UV spectrophotometrically (Jasco V-630, Japan) at 241nm. The phase solubility diagram was constructed by plotting the dissolved CLN against respective concentrations of HP-β-CD. The binding constant K_C was calculated from phase solubility diagram using its slope and intercept values using equation,

$$K_C = \text{Slope} / S_o (1 - \text{Slope})$$

Where, K_C is apparent stability constant, which indicates solubility of drug without HP-β-CD.

Preparation of CLN & HP-β-CD complex

CLN-HP-β-CD inclusion complex were prepared by solvent evaporation method. In the present study, CLN and HP-β-CD complex in the different molar ratio 1:1, 1:2, and 1:3. The desired molar weight of CLN and HP-β- CD (dissolved in minimum quantity of methanol individually) was mixed. The resultant solution was evaporated for 15 min 45ºC in rotary vacuum evaporator (Ikea WERKE, RV 06-ML) The resultant film was stored in desiccator at room temperature.

Table 2: Formulation design for preparation of CLN-HP-β-CD inclusion complex

Batch no.	1	2	3
CLN (mg)	200	200	200
HP-β-CD (mg)	200	400	600
Methanol (ml)	30	30	30

Characterization of prepared CLN & HP-β-CD complex UV/VIS spectrophotometer

The absorption spectrum was carried out using a UV spectrophotometer (Jasco V-630, Japan). The absorption spectrum was recorded against the blank solvent prepared in the absence of CLN and HP-β-CD. The scanned area was from 190 to 600 nm for all formulated samples in triplicate.

Differential scanning calorimetry (DSC)

Thermal analysis was carried out using differential scanning calorimetry (Ta Instruments Q2). Thermogram for CLN plain powder, HP-β-CD powder, and the complex were obtained. For calibration of temperature scale and energy Indium was used. Samples were placed in perforated aluminum pans and heated at a scanning rat of 10^0 C/min for 35 to 300 ^{0}C under nitrogen purge gas flow rate of 25 ml/min.

Drug polymer compatibility studies (FTIR)

FT-IR spectra in region of 400-4000 cm^{-1} for samples were obtained using a FT-IR spectrometer (Jasco 4100, Japan). CLN, HP-β-CD and complex samples were previously grounded and mixed with KBr. And scan the spectra.

Powder X-ray diffraction (XRD)

XRD patterns of raw materials and the prepared inclusion complex were performed at room temperature with a Y-2000 Automated X-ray diffractometer system (Rigaku Miniflex). Monochromatic Cu $K_ć$-radiation was obtained with a Nickel-

filtration. The patterns were recorded on a quartz plate at a tube voltage of 30 kV and a current of 20 mA over a 2 range of 5–45ºC using a step size of 0.06 at a scan speed of 1 sec/step. The peak intensities and 2 values of inclusion complex patterns were compared to those of the physical mixture in order to evaluate the physical form of CLN in the samples.

***In vitro* dissolution studies**

This study was carried out by using a dissolution apparatus. Formulated complexes were tested at 37± 0.5^0c and 75 rpm in 600 ml Phosphate buffer pH 6.8. Solution by using USP type II apparatus. The sample were withdrawn at 5, 10, 15, 30 and 60 min respectively. Withdrawn samples were passed through membrane filter. The filtrates were analyzed by UV spectroscopy at 241 nm. On the basis of UV absorption results dissolution rate of CLN was determined.

3. Results & Discussion

Phase Solubility

CLN aqueous solubility was observed to be 0.0335 mg/ml; therefore, CLN can be defined as practically insoluble drug according to USP. Solubility of CLN alone and in the presence of different dilutions of HP-β-CD is graphically represented in following fig.2. The solubility of CLN increased as a function of HP-β-CD concentrations due to micellar solubilization. Solubility of CLN in presence of 2% w/v HP-β-CD was increased up to 5.29 mg/ml corresponding to 157.566-fold increase, indicating excellent affinity between CLN and HP-β-CD to form a molecular dispersion as per figure 2.

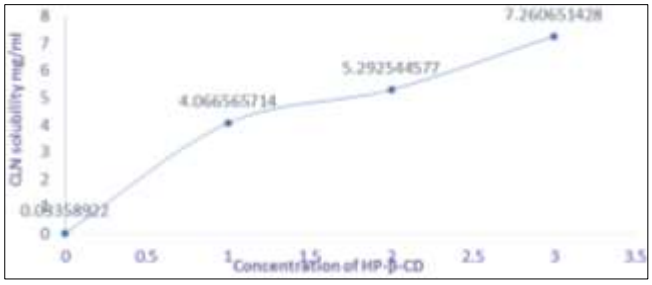

Fig 2: Determination of Phase solubility of CLN with HP-β-CD

Differential Scanning Calorimetrical Studies (DSC)

When CLN was entrapped in a cavity of HP-β-CD and dispersed in the polymer their physicochemical characteristics might change from natural structure. In general, there are boiling, melting and sublimation point could shift to a different temperature or disappeared. Therefore, DSC thermograms was used to evaluate phase transformation of CLN, HP-β-CD and inclusion complex are depicted in Figure 3. Pure CLN showed a sharp, single endothermic peak of 112.75°C corresponding to the melting point of CLN confirming its crystallinity. Although, the thermogram of HP-β-CD showed broad endothermic peak between 46.48 to 97.86ºC, the peak emerged corresponds to the release of water of HP-β-CD cavity. The inclusion complex shows complete disappearance of cilnidipine and some shifts of HP-β-CD peaks were detected, suggesting some interactions between these complexes. The DSC results indicated that CLN was successfully included into the cavity of HP-β-CD in complex inclusion. These thermal changes signify formations of inclusion complex through molecular interactions between the CLN and HP-β-CD.

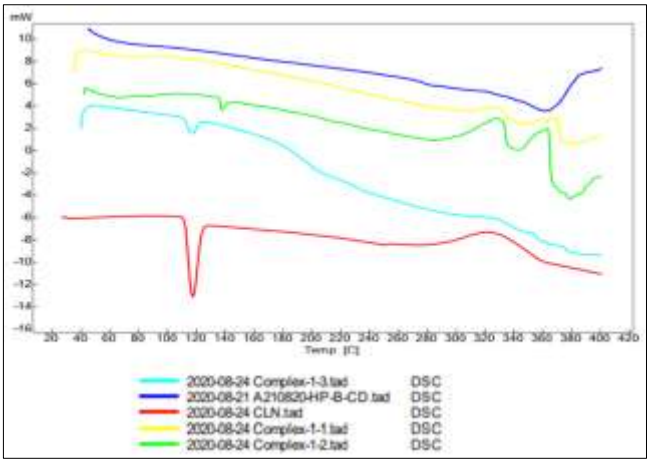

Fig 3: DSC thermograms of CLN plain powder, HP-β-CD and CLN complex

Drug polymer compatibility

The interaction between CLN and HP-β-CD in the complex was visualized by IR spectroscopy. Pure CLN IR spectra showed an intense well-defined characteristic absorption peak lay in the N-H stretch (3290cm^{-1}) vibration of secondary amine and C=O stretch (1692 cm^{-1}), C-O-C stretching peaks at 1272 cm^{-1}. In the addition, the distinctive absorption bands at 1648 cm^{-1} and 1621cm^{-1} denoted the C=C stretch and other sharp absorption peaks at 1522 cm^{-1} and 1347 cm^{-1} attribute to the existence of NO_2. In the spectrum of HP-β-CD, the most characteristic peak showed in the aromatic C-H stretch (2972.62cm^{-1}) and O-H stretch (3351.68cm^{-1}) and C=O stretch (1653.66cm^{-1}). The spectrum of inclusion complex of ratio 1:1 was equivalent to the simple combination of the CLN and HP-β-CD. Some characteristic absorption peaks of CLN at 3290 cm^{-1}(N-H stretch), 1648 cm^{-1}(C=C), 1522 cm^{-1} (NO_2) was observed, which suggesting that the natural structure of CLN still existed without any interactions with HP-β-CD. For the ratio 1:2 and 1:3 complex, this complex spectrum was similar to that of the HP-β-CD and the characteristic peak of CLN was completely disappeared. Moreover, there was no additional peak observed in the spectrum of complex inclusion so there is no interaction between drug and polymer. The FTIR results and were corresponding to the DSC and PXRD results, which indicating CLN was included in the cavity of HP-β-CD. Comparative study of formulation and starting materials is described in detail in table 3-4 and figure 4).

Table 3: Characteristic IR values of CLN, HP-β-CD

S. No.	Functional Group	Observed Frequency cm^{-1}	Functional Group Present	Observed Frequency cm^{-1}
	CILNIDIPINE		**HP-β-CD**	
1.	N-H	3290	C-H	2972
2.	C=O	1602	C=O	1653
3.	C-O-C	1271	O-H	3351
4.	C=C	1648 & 1621		
5.	NO_2	1522 & 1347		

Table 4: Characteristic IR values of CLN- HP-β-CD inclusion complex

S. No.	Functional Group	Observed Frequency cm^{-1}	Functional Group	Observed Frequency cm^{-1}	Functional Group	Observed Frequency cm^{-1}
	1: 1		1:2		1:3	
1	N-H	3290	C-H	2972	C-H	2972
2	C=C	1648 & 1621	C=O	1653	C=O	1653
3	NO_2	1522 & 1347	O-H	3351	O-H	3351

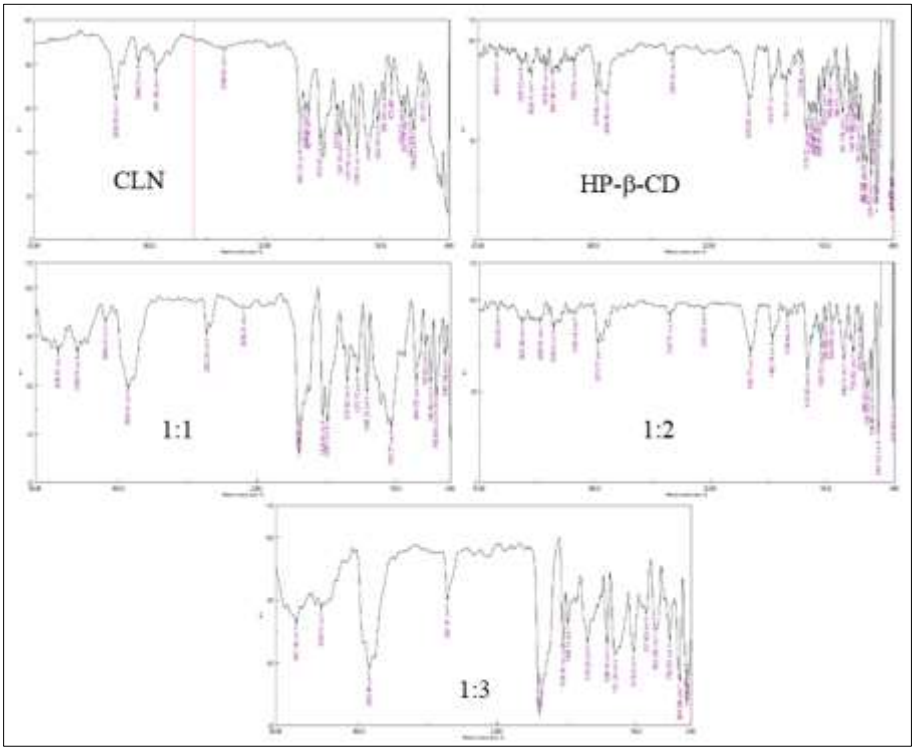

Fig 4: FT-IR spectra of CLN plain powder, HP-β-CD and complex

Powder X-ray Diffraction (XRD)

The physical form of the drug in the inclusion complex, pure CLN and HP-β-CN was examine using XRD the diffraction pattern of CLN displayed some intense and sharp peaks, at numerous 2θ which indicating its crystalline nature. On other hand, the HP-β-CD diffraction pattern was characterized by a typical amorphous nature. Amorphous state is thermodynamically more active and no energy is required to break the crystal lattice in crystalline phase. Therefore, amorphous state is more soluble as compare to crystalline state. In the inclusion complex ratio 1:1 showed the characteristic peak of CLN and the amorphous pattern of HP-β-CD, but the intensities of crystalline peaks in region were significantly less than that of pure CLN. Which indicated the crystalline nature of the drug still maintained. The diffractogram of complex 1:2 and 1:3 showed diffraction pattern with complete absence of numerous characteristic peaks of CLN which indicates amorphous nature of CLN in the inclusion complex. Moreover, the spectrogram of HP-β-CD complex inclusion was not precisely the identical to that of the pure HP-β-CD which indicating some new complex compounds were discovered. The diffraction pattern is depicted in figure 4.

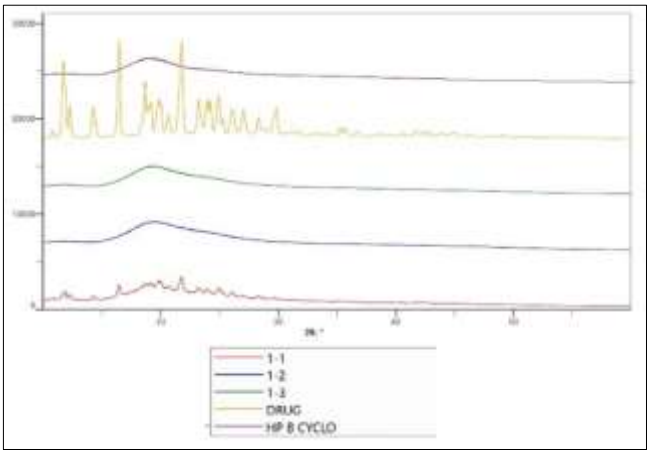

Fig 4: X-ray diffractograms of CLN plain powder, HP-β-CD and complex

Dissolution study

For the dissolution of drug substances solubility plays an important role. It was evident that no dissolution was achieved for pure CLN, with only 20.62% dissolved after 60 min, under the specified dissolution conditions.

The hydrophobic property of the drug prevented its contact with the dissolution medium causing it to float on the surface, and consequently hindering its dissolution[14]. Drug solubility and wettability may be increased by surrounding hydrophilic carriers. As expected, the complex inclusion exhibited markedly faster dissolution than that of pure drug, 88.99% complex of drug was dissolved in 60 min. indicating the remarkable effect of inclusion technique using HP-β-CD in promoting dissolution rates. The *in-vitro* dissolution profiles are depicted in figure 5.

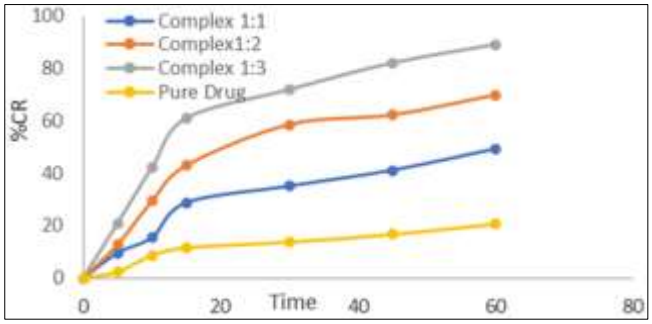

Fig 5: In vitro dissolution profiles of CLN plain powder, HP-β-CD and complex

Conclusion

In this study, CLN solubility was increased to up to 150-fold by integrated complexation with HP-β-CD. X-ray diffractometry and DSC revealed an interaction between CLN and HP-β-CD in the complex. The results suggest that complexation with HP-β-CD was effective for the improvement of CLN bioavailability. CLN-HP-β-CD inclusion complex was successfully prepared by a simple solvent evaporation method which may suitable for industrial manufacturing the products. The CLN-HP-β-CD inclusion complex maintained the antihypertension activity of CLN and significantly enhanced the bioavailability of the orally administered drugs. Furthermore, the dissolution rate of inclusion complex was higher than that of pure drug. All the results suggested that complexation technique was a promising strategy to improve the water-solubility of cilnidipine. This inclusion complex should be regarded as a potential strategy in designing a novel formulation of cilnidipine for hypertension treatment.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this research article.

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