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Investigation of inclusion complex of cilnidipine with hydroxypropyl-β-cyclodextrin

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ABSTRACT

The objective of this study was to improve the water-solubility and photostability of cilnidipine by complexing it with hydroxypropyl-β-cyclodextrin (HP-β-CD or HP-beta-CD). The interactions of cilnidipine and HP-β-CD were characterized by ultra violet–visible (UV/VIS) spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier transformation-infrared (FT-IR) spectroscopy and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy to verify the formation of cilnidipine-HP-β-CD complex inclusion. Moreover, the binding sites in the HP-β-CD structure were also tracked through ^{1}H NMR spectroscopy analysis. All the characterization information proved the formation of cilnidipine-HP-β-CD inclusion complex, and the results demonstrated the superiority of the inclusion complex in dissolution rates and photostability; in addition, the apparent solubility of cilnidipine was increased more than 10,000-fold in the presence of HP-β-CD. The stability constant (1:1) was found to be 50,116 M^{-1}, suggesting a high tendency of the drug to enter the HP-β-CD cavity. These results identified the cilnidipine-HP-β-CD inclusion complex as an effective new approach to design a novel formulation for pharmaceutical application.

1. Introduction

In recent years, inclusion complexes with cyclodextrins (CDs) have been widely used to improve the solubility of water-insoluble drugs, enhance the physical and chemical stability of drugs, eliminate undesired properties of drugs, and improve the bioavailability (Li & Loh, 2008; Van De Manakker, Vermonden, Van Nostrum, & Hennink, 2009). CDs are cyclic oligosaccharides with six, seven or eight D-(+)-glucopyranose units (α, β, and γ-cyclodextrin) attached by α-1,4-linkage (Szejtli, 1998). The most important feature of CDs is that they can trap the lipophilic drug as the guest compounds in their hydrophobic central cavity (Miller, Carrier, & Ahmed, 2007). 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 CDs have been prepared to solve such problems and improve the complexation capacity. Among these CDs, HP-β-CD has been extensively focused on, due to its excellent water-solubility and safety accord by the parenteral route (Misiuk & Zalewska, 2009; Yang, Lin, Chen, & Liu, 2009).

Cilnidipine, a novel dihydropyridine calcium channel blocker, has been reported to exhibit excellent clinical effects on cardiovascular diseases (Kitahara et al., 2004; Minami, Kawano, Makino, Matsuoka, & Takishita, 2000; Narita et al., 2011). A unique pharmacological property for cilnidipine is that it inhibits both L-type and N-type calcium channels in various types of neurons (Kai & Kuzumoto, 2009; Konda, Enomoto, Takahara, & Yamamoto, 2006). Recently, cilnidipine was found to possess much more remarkable advantages compared to traditional calcium-channel blockers; for example, it causes a lower probability of reflex tachycardia than nisoldipine and has less influence on heart rate than nifedipine (Minami, Ishimitsu, Higashi, Numabe, & Matsuoka, 1998a; Minami, Ishimitsu, Kawano, Numabe, & Matsuoka, 1998b). In addition, the renal protective effects of cilnidipine indicate that it has great potency in benefiting hypertensive patients in altering their blood pressures (Kojima, Shida, & Yokoyama, 2004; Morimoto, Yano, Maki, & Iwasaka, 2007). However, the dissolution and oral bioavailability of cilnidipine are not as good as expected, mainly because of its poorly water-soluble property. Moreover, the poor photostability of cilnidipine brings so many conveniences to use and storage.

Until to now, a number of research works about cilnidipine have concentrated on the pharmacology, therapeutics and quantitative analytical methods, however, there is few report focus on developing or characterizing cilnidipine-HP-β-CD inclusion

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complex. Therefore, the main purpose of this research was to utilize the complexation technique with HP-β-CD to improve the solubility and photostability of cilnidipine, and freeze-drying method was employed to prepare inclusion complex of cilnidipine with HP-β-CD (de Araujo et al., 2008; Zeng, Ren, Zhou, Yu, & Chen, 2011). UV/VIS spectroscopy was adopted to evaluate the absorption spectrum of cilnidipine-HP-β-CD complex in aqueous solution. On the other hand, the interactions in the solid state were characterized by DSC, PXRD, FT-IR and ^{1}H NMR, aiming to verify the formation of inclusion complex. Finally, the dissolution behavior and photostability of cilnidipine-HP-β-CD inclusion complex were compared with pure drug to prove the superiority of cilnidipine-HP-β-CD inclusion complex.

2. Experimental

2.1. Materials

Cilnidipine was obtained from Anhui Bengbu Tushan Pharmaceutical Factory (Anhui, China); HP-β-CD was obtained as a gift from Shenyang Pharmacy University Pharmaceutical Factory (Liaoning, China); all other chemicals were of analytical grade, and used without further purification. Distilled water was used throughout the study.

2.2. Preparation

Cilnidipine-HP-β-CD inclusion complex was prepared using a LGJ-10 freeze-dryer (SongYuan Huaxing, China) as follows. Briefly, excess solid cilnidipine was added to HP-β-CD aqueous solutions, followed by the suspension was stirred at room temperature for 24 h. The mixture was then filtered through a 0.45 μm membrane into glass vials. The resulting solution was frozen at −20 °C for 48 h to ensure complete solidification followed by the frozen sample was evaporated under reduced pressure (<10 Pa) to remove water for 12 h to achieve the solid products. Physical mixture (PM) consisting of cilnidipine and HP-β-CD in the same molar ratio were prepared by mixing them together by grinding in a mortar for 10 min, and then freeze-drying procedure were also employed as the same as above.

2.3. Chromatographic conditions

A Shimazu HPLC system was used to determine the concentration of cilnidipine. The HPLC system equipped with two pumps (LC-20AT) and a UV–Vis detector (SPD-20A). Chromatographic analysis was performed on a C18 analytical column (Agela, 5 μm, 4.6 mm × 250 mm, USA). The mobile phase used was acetonitrile: 0.025 mol/l ammonium dihydrogen phosphate solution: cyclohexane = 60:39:1. The flow rate was 1.0 ml/min, the injection volume was 20 μl, and the drug concentration was determined at 240 nm.

2.4. Solubility study

The apparent solubility of cilnidipine was investigated by adding excess cilnidipine into 100 ml of water in the vials. Then the vials containing the mixture were kept in a shaking incubator at 25 °C for 2 days to get equilibrium. The equilibrated samples were filtered with the 0.45 μm millipore filtration to remove the excess drug. Then the drug content was measured by the above-mentioned HPLC method.

2.5. Phase solubility technique

Phase solubility studies were carried out according to the method established by Higuchi and Connors (Higuchi & Connors, 1965). Firstly, excess amounts of cilnidipine were added to aqueous HP-β-CD solutions with different concentrations (ranging from 1 to 10 mM). The samples were stirred for 72 h at room temperature following by the mixtures were filtered through a 0.45 μm membrane filter. The amount of cilnidipine was measured by HPLC method.

2.6. Physical characterization

2.6.1. UV/VIS spectrophotometer

The absorption spectrum was carried out using a UV/VIS spectrophotometer (Pgeneral, China). The absorption spectrum was recorded against the reagent blank prepared in the absence of cilnidipine and HP-β-CD. The scanned area was from 700 nm to 200 nm for each sample.

2.6.2. Differential scanning calorimetry (DSC)

DSC determinations were conducted on a Shimadzu DSC-60 thermal analyzer (Shimadzu Corporation, Japan). Indium was used to calibrate for the temperature scale and energy. Accurately weighted amounts of samples were placed in perforated aluminum pans and heated at a scanning rate of 10 °C/min from 35 to 300 °C, under a nitrogen purge gas flow rate of 25 ml/min.

2.6.3. Powder X-ray diffraction (PXRD)

PXRD patterns of the raw materials, their physical mixture and the prepared inclusion complex were performed at room temperature with a Y-2000 Automated X-ray diffractometer system (Drigc, China). Monochromatic Cu Kα-radiation (λ = 1.5406 Å) was obtained with a Nickel-filtration, and a system of diverging and receiving slides were 1° and 0.2 mm, respectively. The patterns were recorded on a quartz plate at a tube voltage of 30 kV and a current of 20 mA over a 2θ range of 5–45° using a step size of 0.06° at a scan speed of 1 s/step. The peak intensities and 2θ values of inclusion complex patterns were compared to those of the physical mixture in order to evaluate the physical form of cilnidipine in the samples.

2.6.4. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra in the region of 400–4000 cm^{-1} for samples were obtained using a FT-IR spectrometer-8400S (Shimadzu, Japan). The samples were previously grounded and mixed thoroughly with KBr in the ratio of 1:100. Twenty scans over the selected wave number range at a resolution of 4.0 cm^{-1} were averaged for each sample.

2.6.5. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded on an AVANCE III 600 MHz NMR spectrometer (Bruker, Switzerland). Before tests, each sample (cilnidipine, HP-β-CD, physical mixture and inclusion complex) was dissolved in 0.5 ml solutions of CD_3OD (internal reference, δ = 3.31 ppm) to achieve a transparent solution, and equilibrated for at least 48 h before measurement.

2.7. Dissolution studies

Dissolution studies were carried out using a ZRS-8G dissolution tester (Haiyida, China). Test samples were tested at the paddle rotation speed of 75 rpm in 900 ml 0.4% lauryl sodium sulfate solution at 37 ± 0.5 °C. Each formulation contained 5 mg cilnidipine. The samples were withdrawn at 5, 10, 15, 30, 45 and 60 min, and filtered through a membrane filter (pore size 0.45 μm). The filtrates were directly subjected to HPLC for determination.

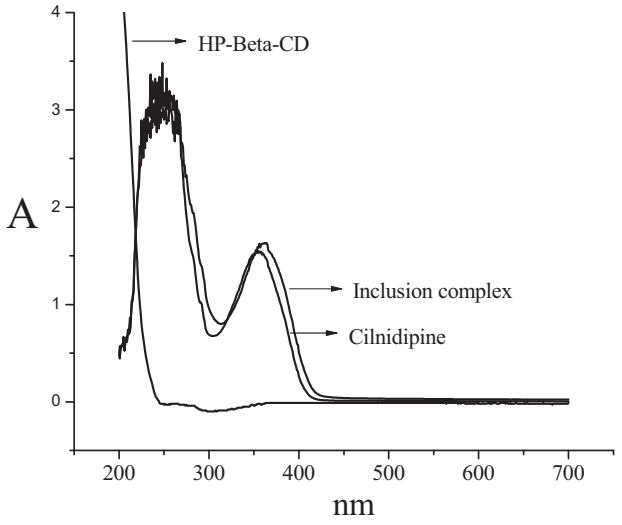

Fig. 1. UV/VIS spectra: Cilnidipine, HP-β-CD and inclusion complex.

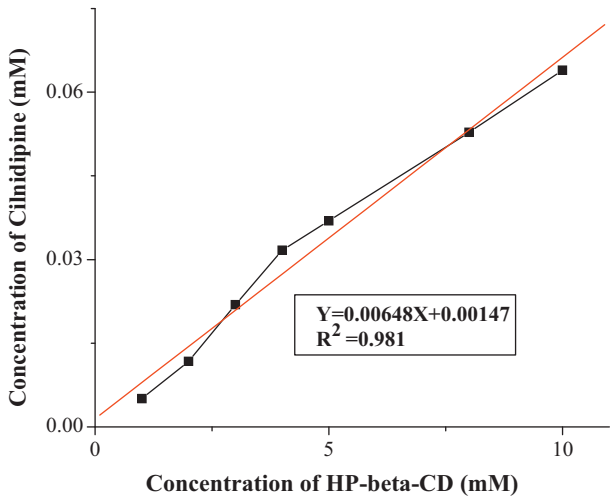

Fig. 2. Phase-solubility diagram for the cilnidipine-HP-β-CD host–guest system at 25 °C.

2.8. Photostability studies

To investigate the influence of HP-β-CD on photodegradation of cilnidipine, the cilnidipine-HP-β-CD inclusion complex was prepared and the concentration of cilnidipine was held constant at 0.5 mg/ml. An ethanol solution of cilnidipine at the same concentration served as a comparison. These formulations were conducted under 4500 lx at 40 °C, and withdrawn at 0, 0.5, 1, 2, 4, 8, 12 and 24 h. The samples were diluted into appropriate concentration, followed by the content of cilnidipine was tracked using the HPLC method described above.

3. Results and discussion

3.1. Absorption spectrum

The influence of complexation on the absorption of cilnidipine was observed by UV/VIS spectrophotometric examination of a 20 μg/ml cilnidipine in 40% HP-β-CD solution and the same concentration of cilnidipine in ethanol solution. The absorption spectra of cilnidipine, HP-β-CD and cilnidipine-HP-β-CD inclusion complex were shown in Fig. 1. The obtained curves showed that HP-β-CD had no absorption within 240–700 nm. The spectrum of cilnidipine-HP-β-CD exhibited two characteristics absorption peaks; in addition, the type and the position were very similar to those of cilnidipine ethanol solution. However, a slightly blue shift was observed in the curve of cilnidipine-HP-β-CD complex, the reason could attribute to the more hydrophilic environment surrounding cilnidipine. These results indicated that the complexation did not have inhibiting effect on absorption of cilnidipine, and the conventional HPLC assay methods of which the detection wavelength was 240 nm can also be applied in determining the concentration of cilnidipine. A validated HPLC method was developed to verify the reliability of this HPLC method. The calibration curve was found to be linear with a correlation coefficient of 0.998. The average extraction recovery was 96.23%, and the within-day and between-day precisions were less than 15%.

3.2. Phase-solubility and association constant

The apparent solubility of cilnidipine-HP-β-CD inclusion complex was found to be 1.84×10^{-3} mol/l, which was over 10,000-fold higher than the solubility of cilnidipine with the absence of HP-β-CD ($S_0 = 1.30 \times 10^{-7}$ mol/l), and the phase-solubility diagram of the cilnidipine-HP-β-CD system was described in Fig. 2. The profile showed the increase on cilnidipine solubility occurred as a linear function of HP-β-CD concentration, which can be classified as the A_L-type complexes defined by Higuchi and Connors (1965). The 1:1 (molar ratio of drug to cyclodextrin) is the most common association type where a single drug molecule is included in the one cavity

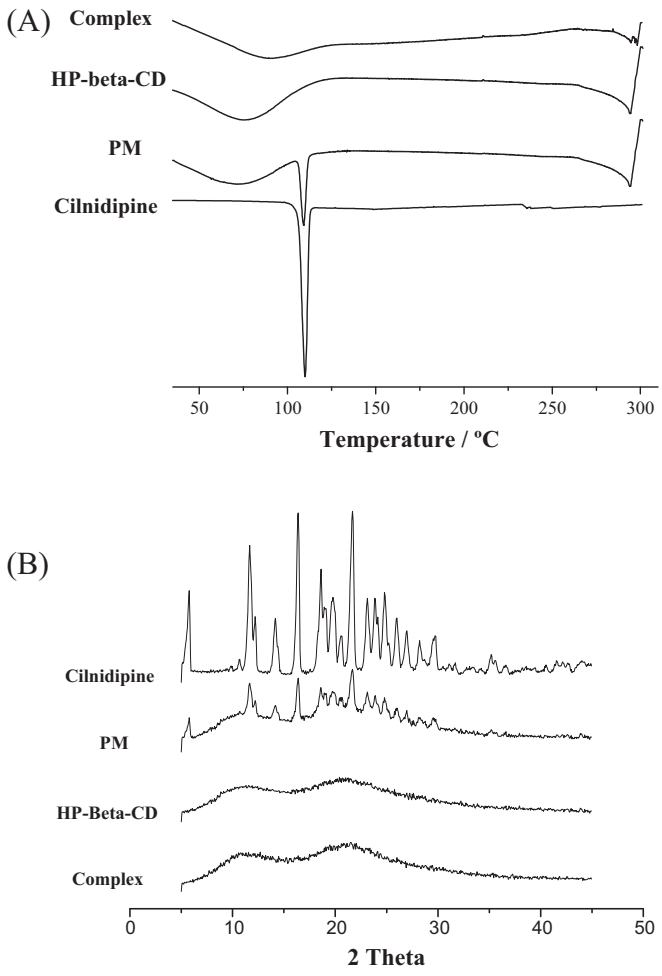

Fig. 3. Characterization of cilnidipine, HP-β-CD, physical mixture and inclusion complex (A: DSC thermograms; B: PXRD spectra).

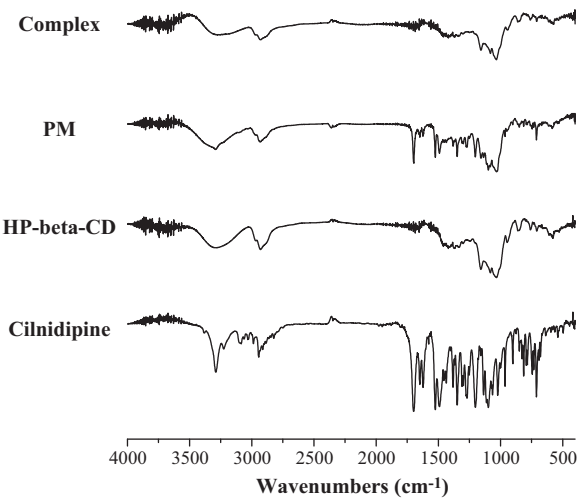

Fig. 4. FT-IR spectra of cilnidipine, HP-β-CD, physical mixture and inclusion complex.

of cyclodextrin molecule, and the apparent stability constant ($K_{1:1}$), was calculated from the linear fit according to the following equation (De Araujo et al., 2007; Higuchi & Connors, 1965; Tommasini et al., 2005): $K_{1:1} = \text{Slope}/[S_0\,(1 - \text{Slope})]$.

The S_0 is the aqueous solubility of cilnidipine and the Slope is the value found in the linear regression. $K_{1:1}$ value is most often found between 50 and 2000 M^{-1}. In our study, the $K_{1:1}$ was found to be 50,116 M^{-1}, suggesting a high tendency of the drug to enter the HP-β-CD cavity.

3.3. Characterization

When guest molecules were included or partly included in cyclodextrin cavities or dispersed in the polymers, their physical characteristics may be different from nature structures. In general, their melting, boiling or sublimation points could shift to a different temperature or disappeared (Chen et al., 2011; Liu & Zhu, 2006). Therefore, the DSC thermograms of cilnidipine, HP-β-CD, physical mixture and inclusion complex were carried out to, and the details were shown in Fig. 3. The unique melting peak of cilnidipine emerged at 109.86 °C, indicating the melting point of cilnidipine. The thermogram of HP-β-CD showed a broad endothermic effect ranging from 35 to 120 °C, and the peak emerged near 80 °C corresponding to the release of water of HP-β-CD. Besides, the irregular peaks near 300 °C could attribute to degradation process of HP-β-CD. For the physical mixture, the drug endothermic peak and the HP-β-CD peaks both presented, indicating the absence of any interactions between cilnidipine and HP-β-CD, and the physical mixture system was still the simple mixture of those components. Concerning the complex inclusion, the complete disappearance of cilnidipine was observed and some shifts of HP-β-CD peaks were also detected, indicating some interactions between these components. The DSC results suggested that cilnidipine was successfully included into the cavity of HP-β-CD in complex inclusion.

Fig. 3 also showed the PXRD patterns of pure drug, HP-β-CD, physical mixture and complex inclusion. The PXRD spectrogram of cilnidipine exhibited characteristic sharp peaks at numerous 2θ, indicating its crystalline nature. Almost the entire characteristic peaks of cilnidipine were observed in the diffraction pattern of physical mixture, but the intensities of crystalline peaks were significantly less than that of intact cilnidipine. This result indicated that the crystalline nature of the drug was still maintained. An important distinction for cilnidipine-HP-β-CD complex inclusion and physical mixture was that no other peaks than those which could assigned to HP-β-CD were observed in cilnidipine-HP-β-CD complex inclusion, implying the crystalline nature was disappeared in complex inclusion. Moreover, the spectrogram of HP-β-CD complex inclusion was not exactly the same to that of pure HP-β-CD, indicating some new complex compounds were formed.

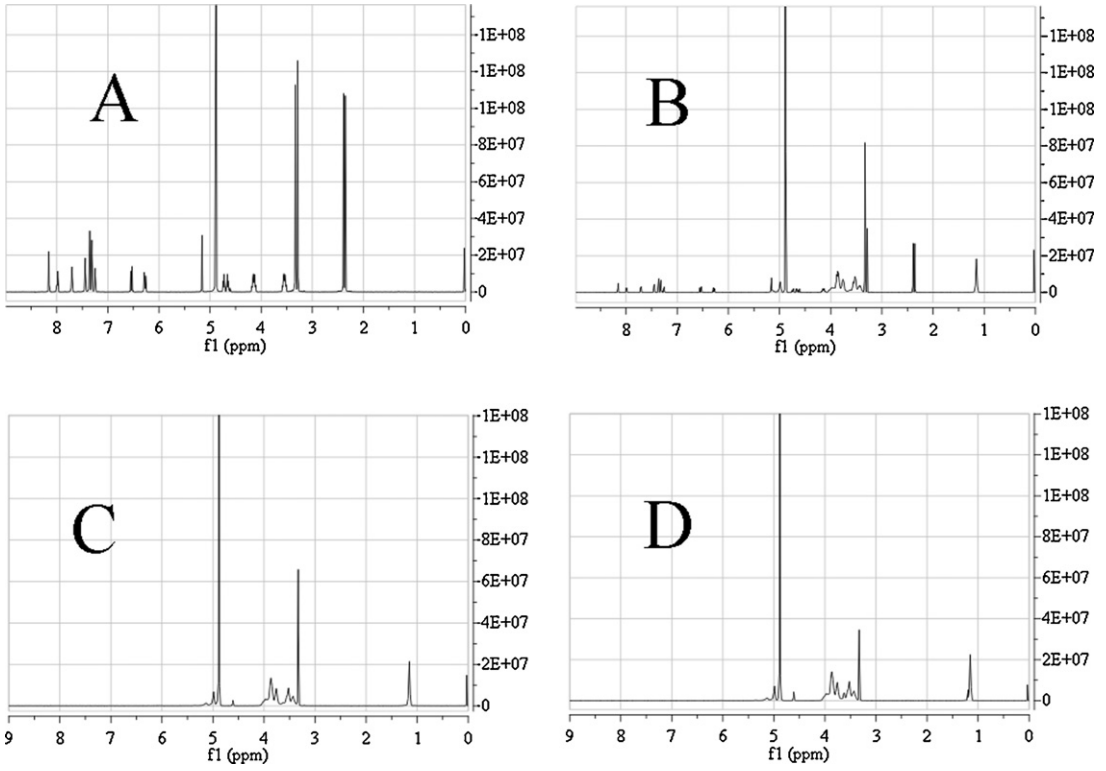

Fig. 5. ^{1}H NMR spectra of cilnidipine (A), physical mixture (B), HP-β-CD (C) and inclusion complex (D).

FT-IR spectra of the pure drug, HP-β-CD, physical mixture and complex inclusion were illustrated in Fig. 4. The most distinct peaks of cilnidipine lay in the N—H stretch (3292 cm^{-1}) and the C=O stretch (1697 cm^{-1}). Moreover, the characteristic absorption bands at 1649 and 1623 cm^{-1} represented the C=C stretch and the absorption peaks at 1524 and 1348 cm^{-1} could attribute to the existence of NO_2. In the spectrum of HP-β-CD, none of the characteristic absorption single peaks emerged in the range 1500–400 cm^{-1}, mainly because of the strong coupling of vibrations from the macrocyclic, caused by neighboring bonds vibrating with similar frequencies. The most characteristic peaks of HP-β-CD lay in the O—H stretch (3400 cm^{-1}), C—H stretch (2930 cm^{-1}) and the C=O stretch (1640 cm^{-1}). The broad peak at 3400 cm^{-1} could attribute to the influence of hydrogen bond. The spectrum of physical mixture was equivalent to the simple combination of the drug and HP-β-CD. Some characteristic absorption peaks of cilnidipine at 3292, 1696, 1648 and 1624 cm^{-1} were easy to be observed, suggesting the natural structure of cilnidipine still existed without any interactions with HP-β-CD. For the complex inclusion, the spectrum was very similar to that of HP-β-CD and the characteristic peaks of cilnidipine were almost entirely disappeared. In addition, no additional peak was detected in the spectrum of complex inclusion, indicating the absence of any chemical reactions between cilnidipine and HP-β-CD. The FT-IR results were corresponding to the DSC and PXRD findings, indicating cilnidipine was included in the cavity of HP-β-CD. In order to investigate more detailed information, the ^{1}H NMR investigations were continued.

The ^{1}H NMR spectra of cilnidipine, HP-β-CD, physical mixture and inclusion complex were shown in Fig. 5. Numerous peaks were found in the spectrum of cilnidipine: 2.3555 (3H, s, C**H**$_3$), 2.3886(3H, s, C**H**$_3$), 3.2843(CD_3OD), 3.3292(3H, s, —OC**H**$_3$), 3.5255–3.5623(2H, m, $CH_3OC\mathbf{H}_2CH_2$—), 4.1224–4.1597(2H, m, $CH_3OCH_2C\mathbf{H}_2$—), 4.6695–4.7293(2H, m, —C**H**$_2$—CH=CH—), 4.8844(D_2O), 5.1576(1H, s, C**H**), 6.2609(1H, dt, —CH_2—C**H**=CH—), 6.5518(1H, d,—CH_2—CH=C**H**—), 7.2488–7.3657(6H, m, Ar**H**), 7.7002(1H, dd, Ar**H**), 7.9805(1H, dd, Ar**H**), 8.1548(1H, s, Ar**H**). All the information was corresponding to the chemical structure of cilnidipine; however, the presence of CD_3OD resulted in the absence of the NH stretch. In the spectrum for HP-β-CD, the signals for H-1, H-2, H-3, H-4, H-5 and H-6 appeared at 4.9860, 3.5216, 3.9686, 3.4199, 3.7614 and 3.8651, respectively, corresponding to some other literatures (Kim et al., 2004). For HP-β-CD, the H-3 and H-5 protons were found to be the most common binding sites in inclusion complexes (Boudad et al., 2001; de Araujo et al., 2008). All these signals were also emerged in the spectrum of physical mixture in reduced shapes. However, the signals relating to the cilnidipine were almost all disappeared in the spectrum of inclusion complex, the reason could attribute to the following factors: (i) the percentage of cilnidipine was quite small in the inclusion complex sample; (ii) the complexation in the cavity could lead to a reduction of signals relating to the guest compounds. Some literatures reported some obvious shifts of the signals relating to guest compounds or CDs could be found in the spectrum of inclusion complex when the guest compounds were included in the CDs. The chemical shifts relating to HP-β-CD were compared between the inclusion complex or physical mixture and HP-β-CD with the absence of cilnidipine; in addition, some details were shown in Table 1 ($\Delta\delta1 = \delta_{complex} - \delta_{HP\text{-}\beta\text{-}CD}$, $\Delta\delta2 = \delta_{PM} - \delta_{HP\text{-}\beta\text{-}CD}$). From Table 1 we could see a significant variation that the H-3 proton of HP-β-CD shifted 0.018 ppm in the spectrum of inclusion complex. However, this distinct shift was not emerged in H-5 proton, and no other obvious shift could be observed in other protons in the comparisons between physical mixture and HP-β-CD. The ^{1}H NMR investigations suggested that cilnidipine was included in the HP-β-CD cavity, and the H-5 proton was found to serve as binding sites between cilnidipine and HP-β-CD in inclusion complex.

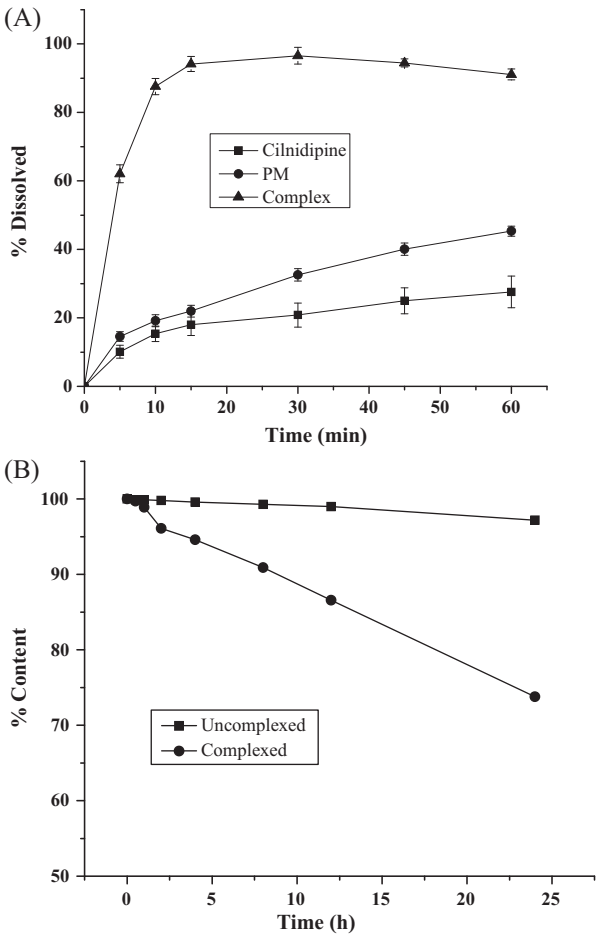

Fig. 6. Dissolution behaviors of cilnidipine, physical mixture and inclusion complex (A) and the degradation rates of the complexed and uncomplexed cilnidipine when conducted under 4500 lx at 25 °C (B).

3.4. Dissolution and solubility results

The power dissolution behaviors of pure drug, physical mixture and complex inclusion were shown in Fig. 6. The dissolution pattern of cilnidipine was found to be very slow, in accordance with its poorly water-soluble property. As expected, the complex inclusion exhibited markedly faster dissolution than that of pure drug and physical mixture, over 90% of the loaded drug dissolved in less than 15 min, indicating the remarkable effect of inclusion technique using HP-β-CD in promoting dissolution rates.

3.5. Photostability studies

Some literatures described that the hydrolytic or photolytic decomposition of guest compounds could be decelerated through the host–guest structure in inclusion complexes (Loftsson & Brewster, 1996). The degradation behaviors of cilnidipine with its

Table 1
Chemical shift change values relating to the signals of HP-β-CD.

	Complex	PM	HP-β-CD	$\Delta\delta1$	$\Delta\delta2$
H-1	4.9871	4.9869	4.9860	0.0011	0.0009
H-2	3.5227	3.5207	3.5216	0.0011	−0.0009
H-3	3.9683	3.9684	3.9686	−0.0003	−0.0002
H-4	3.4202	3.4198	3.4199	0.0003	−0.0001
H-5	3.7596	3.7616	3.7614	−0.0018	0.0002
H-6	3.8660	3.8655	3.8651	0.0009	0.0004

complex were shown in Fig. 6. The uncomplexed cilnidipine ethanol solution showed a fast degradation behavior under the high-light exposure experiment at 4500 lx: over 25% content degraded after 24 h, suggesting the poor photostability of cilnidipine. However, the curve of the cilnidipine in inclusion complex exhibited a different behavior from the cilnidipine with the absence of HP-β-CD. For the cilnidipine in inclusion complex, a lag phase was easy to be found in the figure, and the degradation rate was much slower than that of uncomplexed cilnidipine in the whole period. This remarkable difference proved the protective action of the host-guest system, and the photostability of cilnidipine was improved through the complexation technique with HP-β-CD.

4. Conclusion

In this study, the results of DSC, PXRD, FT-IR and ^{1}H NMR all proved the formation of cilnidipine-HP-β-CD inclusion complex. The details of ^{1}H NMR indicated the binding site of the inclusion complex mainly located in the H-5 in the structure of HP-β-CD. The stability constant of the inclusion complexes was found to be 50,116 M^{-1} for 1:1 cilnidipine-HP-β-CD inclusion complex. The solubility and dissolution behavior of cilnidipine was significantly improved through this technique. Moreover, in the photostability investigations, the cilnidipine in complexation showed a more stable behavior than cilnidipine in the absence of HP-β-CD. All the results suggested that complexation technique was a promising strategy to improve the photo-protective effects and water-solubility of cilnidipine. This inclusion complex should be regarded as a potential strategy in designing a novel formulation of cilnidipine for hypertension treatment.

Declaration of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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