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ORIGINAL ARTICLE

Inclusion complexes of fluorofenidone with β-cyclodextrin and hydroxypropyl-β-cyclodextrin

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Abstract

Background: Fluorofenidone is a novel antifibrotic drug and its aqueous solubility is low. *Aim*: This study was to prepare and characterize inclusion complexes of fluorofenidone (AKF-PD) with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD). *Method*: The AKF-PD/cyclodextrins (CDs) inclusion complexes were prepared by coprecipitation and freeze-drying, respectively. The solubility enhancement of AKF-PD was evaluated by phase solubility method. Inclusion complexation in solid phase was studied by X-ray diffraction (XRD) and differential thermal analysis (DTA). The dissolution profiles of AKF-PD/CDs inclusion complexes were investigated and compared with those of their physical mixtures and AKF-PD alone. *Results*: The phase solubility diagrams of AKF-PD with β-CD and HP-β-CD were of A_L-types, and the solubility of AKF-PD could be increased by 51.5% for β-CD at 0.014 M and 794.0% for HP-β-CD at 0.254 M. The results from XRD and DTA suggested that AKF-PD could form inclusion complex with β-CD or HP-β-CD. The dissolution rate of AKF-PD from the inclusion complexes was much more rapid than AKF-PD alone. *Conclusions*: The formulation of AKF-PD/CDs inclusion complexes showed superior performance in improving dissolution properties of AKF-PD.

Key words: *β-Cyclodextrin; dissolution rate; fluorofenidone; hydroxypropyl-β-cyclodextrin; inclusion complex; phase solubility*

Introduction

Fluorofenidone (AKF-PD), 5-methyl-1-(3-fluorophenyl)-2-[1H]-pyridone (Figure 1), is a novel antifibrotic drug, and its antifibrotic effect has been validated by several animal models and cellular experiments[1]. AKF-PD can inhibit the cells produced by various extracellular matrixes (ECM), therefore, it can be used as an active ingredient for the antifibrotic agent in organs and tissues[2]. However, the aqueous solubility of AKF-PD is low. Thus, the dissolution process is the rate-limiting step for the AKF-PD absorption. We should take measures to improve its solubility and dissolution rate.

Natural cyclodextrins (CDs) are cyclic oligosaccharides, containing six (α-CD), seven (β-CD), or eight (γ-CD)α-l, 4-linked glucopyranose units, with hydrophilic outer surfaces and a hydrophobic cavity[3]. CDs are able to form inclusion complexes with several poorly water-soluble compounds. Upon inclusion, the water solubility of the guest can increase as well as its bioavailability[4–6]. Among the CDs, β-cyclodextrin is the most useful compound for drug complexation. However, its aqueous solubility is rather low (about 1.8% w/v, at 25°C), which led to a search for more soluble derivatives of CDs. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a hydroxypropylated derivative of β-CD, whose solubility in water is more than 50% (w/v) at 25°C. It has been widely studied as a complexing agent for many pharmaceuticals, such as parenteral and oral applications[7,8]. Complexation of drugs with β-CD and HP-β-CD has been shown to increase their solubility, dissolution rate, and stability in aqueous solutions[9].

The aim of this study was to improve the apparent solubility and dissolution rate of AKF-PD in aqueous solution through the formation of inclusion complexes. The inclusion complex of AKF-PD with β-CD was prepared by coprecipitation, and the other kind of inclusion complex of AKF-PD with HP-β-CD was prepared by freeze-drying methods because of the high solubility of

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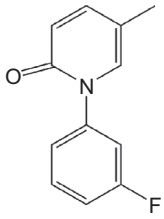

Figure 1. Chemical structure of AKF-PD, 43 × 32 mm (300 × 300 DPI).

HP-β-CD in water. The effect of CDs concentration on the solubility of AKF-PD was determined according to phase solubility studies. The dissolution profiles of AKF-PD/CDs inclusion complexes were investigated and compared with those of their physical mixtures and AKF-PD alone. In addition, X-ray diffraction (XRD), differential thermal analysis (DTA) methods were carried out to verify inclusion complexes formation in the solid state.

Materials and methods

Materials

AKF-PD standard (purity: 99.38%) was synthesized by the Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Central South University (Changsha, China). β-CD was purchased from Qianhui Fine Chemical Co. Ltd. (Zibo, China). 2-HP-β-CD was purchased from Deli Biochemical Co. Ltd. (Xian, China). All other reagents and solvents were of analytical grade, and double distilled water was used throughout the study.

Determination of AKF-PD by UV/visible spectrophotometry

Standard solutions of AKF-PD ranging from 2.884 to 28.840 μg/mL were prepared in distilled water. The absorbencies of these solutions were measured using UV/visible spectrophotometry at 311 nm (UV-2450, JAP). The response fitted a linear regression model within the calibration range (r^2 = 0.99998). The recovery is 99.2% and the precision is 0.32% (n = 6). Additionally, the presence of CD did not interfere the UV absorbance of AKF-PD at 311 nm.

Phase solubility studies

Phase solubility studies were carried out in water according to the method described by Higuchi and Connors[10]. Briefly, excess amounts (ca. 200 mg) of AKF-PD were added to 10 mL of aqueous solution containing various concentrations of CDs (0–0.014 M for β-CD and 0–0.254 M for HP-β-CD). Then, the suspensions were mechanically shaken (LSHZ-300, Huamei Biochemistry Instrument, Taicang, China) at 25°C for 48 hours. After equilibrium attainment, the samples were filtered through 0.45-μm cellulose acetate membrane filters and properly diluted. The absorption value of AKF-PD was determined spectrophotometrically (UV-2450, JAP) at 311 nm. The apparent stability constants (Ks) were calculated from the slope of the phase solubility diagrams according to the following Equation (1), where S_0 is the solubility of drug in water

$$K_s = \frac{\text{slope}}{S_0(1-\text{slope})} \quad (1)$$

Preparation of physical mixture of AKF-PD and cyclodextrins

Physical mixtures of AKF-PD and CDs (molar ratio 1:2) were obtained by simple blending in a glass mortar and were mixed uniformly.

Preparation of AKF-PD/cyclodextrin inclusion complexes

The inclusion complex of AKF-PD with β-CD was prepared by coprecipitation, and the other kind of inclusion complex of AKF-PD with HP-β-CD was prepared by freeze-drying methods because of the high solubility of HP-β-CD in water.

AKF-PD/β-CD inclusion complex by coprecipitation
AKF-PD and β-CD (molar ratio 1:2) were accurately weighed. AKF-PD was dispersed into saturated β-CD aqueous solution and mixed in a laboratory stirrer for 2 hours at 60°C. Then, the solution was cooled down gradually and kept stirring at ca. 10°C to stay overnight. Afterwards, the precipitated product was filtered out and dried under vacuum at 40°C.

AKF-PD/HP-β-CD inclusion complex by freeze-drying
AKF-PD and HP-β-CD (molar ratio 1:2) were accurately weighed. AKF-PD was dispersed into HP-β-CD aqueous solution (20%, w/v), and the obtained suspension was stirred magnetically at 70°C. After the dissolution was completed, the solution was cooled down to room temperature and then lyophilized with a vacuum freeze dryer (Model Lyo-0.5, Tofflon Science & Technology Co. Ltd., Shanghai, China).

Characterization of AKF-PD/cyclodextrin inclusion complexes

X-ray diffractometry
The powder samples were packed in the X-ray holder prior to analysis. X-ray powder diffraction patterns were

recorded on a Rigaku-D/MAX-2500PC diffractometer using Ni-filtered, Cu-Kα radiation, a voltage of 40 kV, and a 250 mA current. The scanning rate employed was $0.02°s^{-1}$ over a 2θ range of 5–70°. The XRD traces of AKF-PD raw material, CDs, AKF-PD/CDs inclusion complexes and their physical mixtures at the same molar ratio were compared with one another in terms of peak position, relative intensity, and the presence or lack of peaks in certain regions of 2θ values.

Differential thermal analysis

DTA curves of AKF-PD raw material, CDs, AKF-PD/CDs inclusion complexes and their physical mixtures were measured with a DTA instrument (Model TAS 100, JAP). Each sample (5–9 mg) was accurately weighed and heated in an aluminum pan at a rate of 10°C/min between 30°C and 500°C temperature range under an air flow. The DTA curves were compared with one another regarding to peak position, peak shifting, and the presence or lack of peaks in certain temperature values.

Dissolution rate studies

In vitro dissolution studies of AKF-PD, CDs, AKF-PD/CDs inclusion complexes and the physical mixtures were carried out in a dissolution apparatus (ZRS-8, Radio Factory, Tianjin University, China) using the paddle method at 37°C ± 0.5°C, rotating at 50 rpm. Hundred milligrams of AKF-PD, or its equivalent in physical mixture or inclusion complex, was added to 250 mL of distilled water. Two milliliters of solution was withdrawn and replaced with the same volume of fresh medium at 2, 5, 8, 10, 15, 20, 30, and 45 minutes. The solution was immediately filtered (0.45 μm pore size), properly diluted, and drug content was determined spectrophotometrically at 311 nm. A correction of drug concentration was made in consideration of replacing with fresh medium after sampling.

Results and discussion

Phase solubility studies

The phase solubility behavior of AKF-PD in CDs solution was shown in Figures 2 and 3. The diagrams showed that the aqueous solubility of AKF-PD increased in a linear manner as a function of β-CD or HP-β-CD concentration, which resulted in A_L-type phase solubility diagrams according to Higuchi and Connors[10]. The apparent stability constants (*K*s) were calculated according to Equation (1) from the totally linear solubility diagrams.

In the case of β-CD, the solubility of AKF-PD could be increased by 51.5% at the β-CD concentration of 0.014 M and the *K*s were obtained to be 82.53 M^{-1}. Concerning the

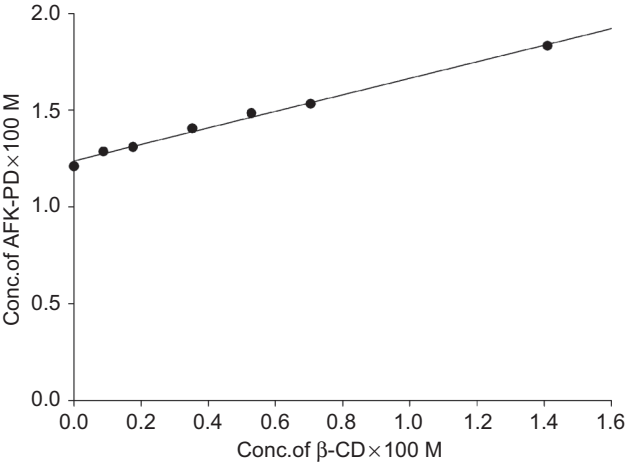

Figure 2. Phase stability diagram of AKF-PD/β-CD system in water, 54 × 45 mm (300 × 300 DPI).

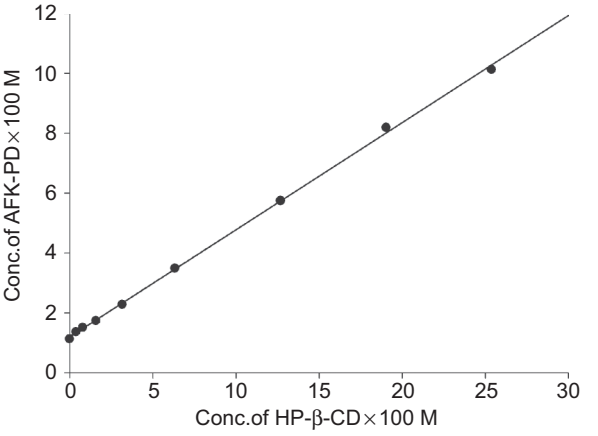

Figure 3. Phase solubility diagram of AKF-PD/HP-β-CD system in water, 43 × 32 mm (300 × 300 DPI).

HP-β-CD, the solubility of AKF-PD could be increased by 50.3% at the HP-β-CD concentration of 0.014 M, which was close to that with β-CD. However, because of the high solubility of HP-β-CD, the solubility of AKF-PD was increased by 794.0% for HP-β-CD at 0.254 M. The *K*s were obtained to be 49.06 M^{-1}. By comparison, the complexation of AKF-PD with HP-β-CD is relatively less than that with β-CD, probably due to the presence of substituent hydroxypropyl groups in HP-β-CD, which may hamper inclusion of some guest molecules into the CD cavity by steric hindrance[11,12].

Preparation of inclusion complexes

The solid products in 1:1, 1:2, and 1:3 drug : CD ratio have ever been prepared. The results from DTA, XRD indicated the complete formation of complex in 1:2 and 1:3 ratio, but the partial formation of complex in 1:1 ratio,

which seems to be inconsistent with the phase solubility study in this article. This was possibly because of the rather low stability constants. To obtain the complete formation of complex, 1:2 drug : CD ratio was finally chosen.

X-ray diffractometry

Figure 4 showed the XRD patterns of AKF-PD, CDs, AKF-PD/CDs inclusion complexes as well as their physical mixtures. The AKF-PD and β-CD diffraction patterns that are shown in Figure 4a and b displayed intense peaks, which was an indicator of their crystalline character. In contrast, HP-β-CD shown in Figure 4e is amorphous lacking crystalline peaks. Some drug crystallinity peaks were still detectable in the physical mixtures shown in Figure 4d and g.

Compared to the diffraction patterns of pure AKF-PD and HP-β-CD, the diffractogram of the inclusion complex shown in Figure 4f was completely similar to that of the amorphous HP-β-CD. The diffraction pattern of AKF-PD/β-CD inclusion complex shown in Figure 4c showed less intense peaks, but the disappearance of the prominent crystalline peak of AKF-PD situated at 8.6° (2θ) was clearly observed. These results confirmed that AKF-PD was no longer present as a crystalline material which indicated the interaction of AKF-PD and cavity of CDs[13].

Differential thermal analysis

Thermal analysis provided additional evidence that inclusion complexes were formed. When guest molecules were embedded in CD cavities or in the crystal lattice, their melting, boiling, or sublimation point generally shifted to a different temperature or disappears within the temperature range where CD decomposes[14].

The DTA thermogram of AKF-PD exhibited a sharp endothermal peak at 136.13°C (Figure 5a), indicating the melting point of AKF-PD. In the case of β-CD (Figure 5b), due to a dehydration process, β-CD presents a broad endothermic peak around 80°C. Additionally, to a decomposition process, there were two continuous broad endothermic peaks at 298.20°C and 320.81°C, respectively. The trace of HP-β-CD (Figure 5e) also showed two continuous broad endothermic peaks at 317.97°C and 336.49°C, respectively, corresponding to the decomposition of HP-β-CD[15].

In the DTA curves of the AKF-PD/CDs inclusion complexes (Figure 5c and f), the endothermal peak of AKF-PD was not observable, indicating the interaction of AKF-PD and cavity of CDs, which in turn led to an almost complete loss of crystallinity of this binary system. Compared to the physical mixture of AKF-PD and β-CD, this endothermal peak was clearly visible although less intense. However, the thermal curve of the physical mixture of AKF-PD and HP-β-CD showed approximately the same thermal behavior (Figure 5g). Similar result was reported previously[16]. The disappearance of the drug-melting peak of physical mixture might be because of the drug amorphization during the DTA run in the presence of amorphous carrier. Moreover, the relative intensity of the continuous endothermic peaks was different between the AKF-PD/HP-β-CD inclusion complex and the physical mixture.

Dissolution studies

Figure 6 showed the dissolution profiles of AKF-PD, its inclusion complexes, and physical mixtures. It was evident that both the complexes and the physical mixtures exhibit faster dissolution rate than AKF-PD. The increase in the dissolution rate of AKF-PD when physically mixed

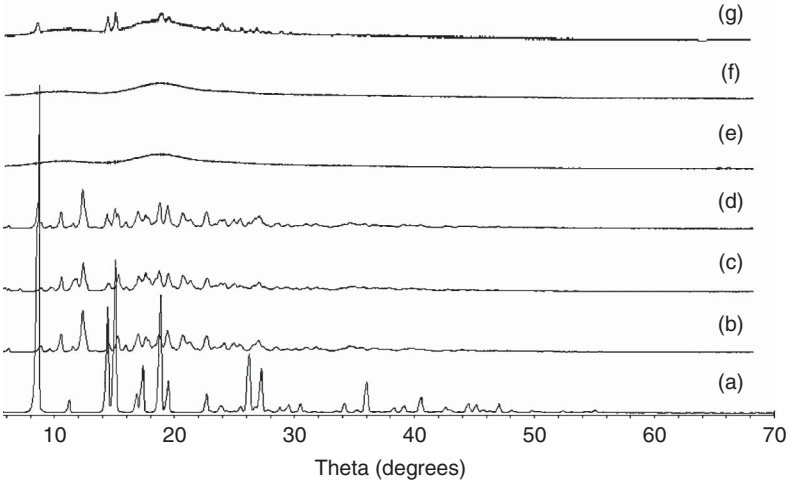

Figure 4. XRD patterns of (a) AKF-PD, (b) β-CD, (c) AKF-PD/β-CD inclusion complex, (d) physical mixture of AKF-PD and β-CD, (e) HP-β-CD, (f) AKF-PD/HP-β-CD inclusion complex, and (g) physical mixture of AKF-PD and HP-β-CD, 74 × 45 mm (300 × 300 DPI).

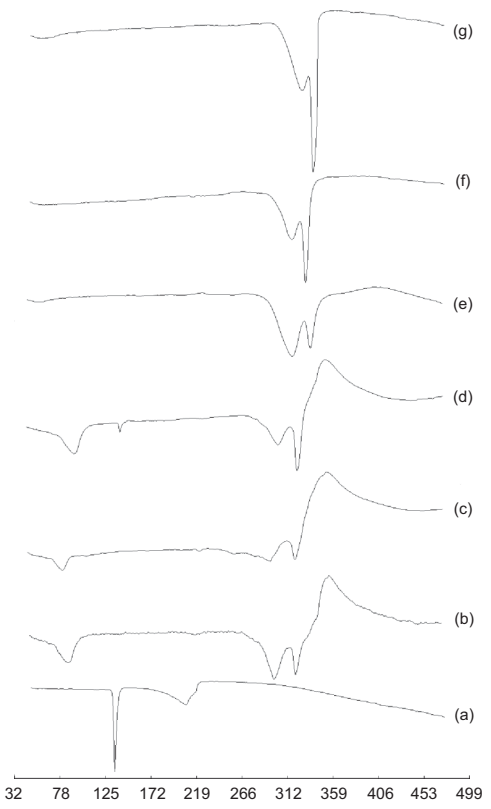

Figure 5. DTA thermograms of (a) AKF-PD, (b) β-CD, (c) AKF-PD/β-CD inclusion complex, (d) physical mixture of AKF-PD and β-CD, (e) HP-β-CD, (f) AKF-PD/HP-β-CD inclusion complex, and (g) physical mixture of AKF-PD and HP-β-CD, 54 × 49 mm (300 × 300 DPI).

with CDs was possibly because of the solubilizing effect of the CD and also to improve wettability of the drug[17]. In situ formation of readily soluble complexes was also possible[18].

In the case of AKF-PD/HP-β-CD inclusion complex, the amount dissolved after 2 min was more than 90 wt %. The very high increase of the drug dissolution rate in the case of inclusion complexes might be due to several reasons: the formation of soluble inclusion complex, amorphization of the drug and consequently solubility increase, better wettability, and reduction of particle size[19]. The increase in drug dissolution from AKF-PD/HP-β-CD systems was higher than from the corresponding ones with β-CD, showing the importance of the proper choice of the carriers. The better effectiveness of HP-β-CD was the result of its greater water solubility, higher wetting, and complexing ability to AKF-PD although its complexing ability was lower than that of β-CD.

Conclusions

The solid inclusion complex of AKF-PD with β-CD was prepared by coprecipitation while that with HP-β-CD was obtained by freeze-drying. The results, from both DTA and XRD studies, suggested that AKF-PD could form 1:2 molar ratio inclusion complex with either β-CD or HP-β-CD. The phase solubility of AKF-PD with β-CD and HP-β-CD both showed A_L-type diagrams, and the solubility of AKF-PD could be increased by 51.5% for β-CD at 0.014 mol/L and by 794.0% for HP-β-CD at 0.254 mol/L, respectively. The inclusion complex of AKF-PD/HP-β-CD was

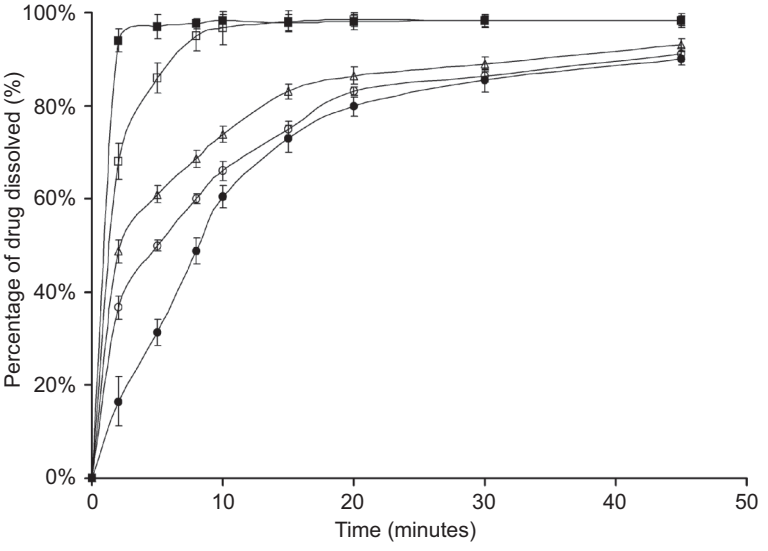

Figure 6. The dissolution profiles of (●) AKF-PD, (○) physical mixture of AKF-PD and β-CD, (Δ) AKF-PD/β-CD inclusion complex, (□) physical mixture AKF-PD and HP-β-CD, and (■) AKF-PD/HP-β-CD inclusion complex, 37×26 mm (300×300 DPI).

completely amorphous and displayed the best dissolution performance. Additionally, the increase in drug dissolution from AKF-PD/HP-β-CD systems was higher than from the corresponding ones with β-CD.

In conclusion, our results demonstrated that the formulation of AKF-PD with CDs significantly could improve its solubility, and the resultant product could improve the dissolution properties of AKF-PD.

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Declaration of interest: The authors report no conflicts of interest.

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