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Investigation of a fenofibrate-hydroxypropyl-β-cyclodextrin system prepared by a co-grinding method

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Considering the poor water solubility and the low oral bioavailability in humans, the complex formation of fenofibrate (FNB) with hydroxypropyl-β-cyclodextrin (HP-β-CD) in aqueous solution was studied. The effect of temperature on the complexation was examined and thermodynamic parameters of the complexation process such as Gibbs free energy change (ΔG), enthalpy change (ΔH) and entropy (ΔS) change were also determined. Meanwhile, the solid dispersion of FNB with HP-β-CD was prepared and characterized by X-ray diffractometry and Fourier IR spectroscopy. The experimental results indicated that a 1:1 molar ratio complex of FNB with HP-β-CD could form in aqueous solution and the complexation was exothermic and enthalpy-driven process. The FNB-HP-β-CD solid dispersion presented a remarkable improvement in the solubility and dissolution rate of the drug. This might be attributed to the amorphous state, the enhanced wettability and as well as the complex formation of the drug with HP-β-CD in aqueous solution.

1. Introduction

Fenofibrate (FNB) is an oral fibrate lipid lowering drug, which could markedly reduce elevated plasma concentrations of triglycerides, low density lipoprotein (LDL) and total cholesterol (Guay 1999; Milionis et al. 2000; Shi et al. 2005). It is clinically used to regulate the metabolism of lipids (Adkins et al. 1997). As the compound is practically insoluble in water (Palmeiri et al. 1996), the oral bioavailability of the drug in humans is relatively low (Munoz et al. 1994; Tang et al. 2009). Presently, many methods or techniques such as micronization, solid dispersion, cyclodextrin complexation, liposomes, nanosuspensions/ nanoparticles, self-assemblies and self-microemulsifying drug delivery system (SMEDDS), etc have been tried to improve the solubility, dissolution and therefore oral bioavailability of the drug (Vogt et al. 2008; Mochalin et al. 2009; Sheu et al. 1994; Patel et al. 2006, 2007; Santa et al. 2005; Hanafy et al. 2007; Chen et al. 2009).

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight D-glycopyranose uinits (α-, β-, γ-cyclodextrin) linked by a (1–4) glycosidic bonds. They have a torus-shaped, apolar, electron rich hydrophobic cavity, with internal cavities of 0.5, 0.6, and 0.8 nm, respectively. The hydroxyls of this macromolecule oriented to the exterior create sites available for hydrophobic interactions. Initially, the CD cavity is occupied by water molecules. The presence of hydrophobic molecules in the aqueous media leads to noncovalent complex formation through displacement of the included water molecules by the hydrophobic ones (Szejtli 1998). The complex formation usually results in a modulation of the physicochemical and pharmaceutical properties of guest molecules, such as increased solubility and dissolution rate, improved chemical stability and bioavailability, reduced toxicity and irritation, controlled rate release and so on (Stella et al. 2008). A derivative of β-CD, HP-β-CD has gained considerable attention because of its greater aqueous solubility and higher safety (Pitha et al. 1986). It has been extensively used in improving the solubility and dissolution rate of various poorly water-soluble hydrophobic drugs (Zia et al. 2001). CDs such as α-, β-, γ-CD, methyl-β-CD (Me-β-CD), dimethyl-β-CD (DM-β-CD), hydroxyethyl-β-CD (HE-β-CD), etc have been reported to be able to form complexes with FNB (Patel et al. 2006; Palmieri et al. 1997). The aim of this work was to investigate the complexation behavior of FNB with HP-β-CD in aqueous solution and to further study thermodynamic properties involved in the process. Meanwhile, a FNB-HP-β-CD solid dispersion was prepared and its solubility and dissolution behavior were also studied in this paper.

2. Investigations, results and discussion

2.1. UV absorption studies

The effect of HP-β-CD on the absorption spectrum of FNB is shown in Fig. 1. The absorption intensity of the drug gradually decreased with an increase in the concentration of HP-β-CD, and change in the absorbance was most remarkable at the maximum absorption position (approximately 290 nm) of the drug, indicating the complex formation of FNB with HP-β-CD in aqueous solution (Gu et al. 2005). These changes in the absorption intensity were assumed to result from changes in the solvent microenvironment upon complexation of the solute. The reduced peak absorption intensity might be the result of the transference of guest molecules from water to the CD cavity (Arias et al. 1997). This is reasonable because there are no proton donating groups inside the cavity of the CD molecule (Ismail 1991).

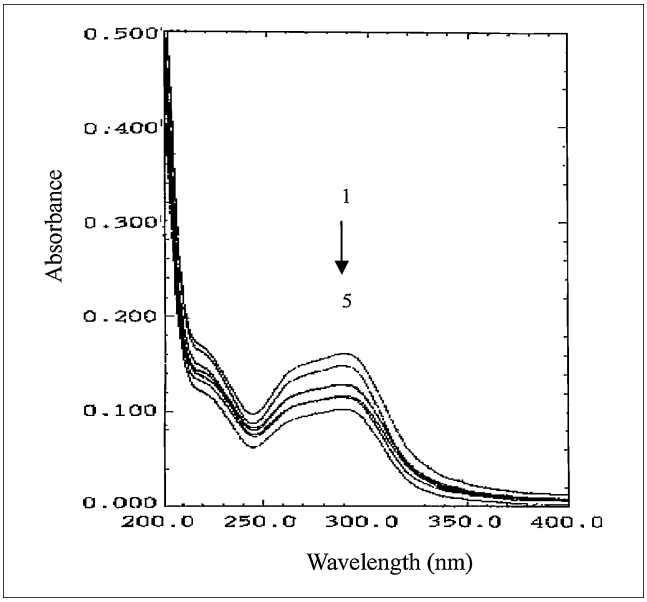

Fig. 1: Effect of HP-β-CD on UV spectra of FNB in water. The concentration of HP-β-CD is 0–8.8 × 10^{-4} m

2.2. Phase solubility studies

As shown in Fig. 2, the solubility of FNB increased linearly as a function of HP-β-CD concentration. The phase solubility diagram follows an AL-type according to Higuchi and Connors' classification, suggesting the formation of a soluble complex of 1:1 molar ratio (Higuchi et al. 1965). Solubility of FNB was increased by about 20-fold at 50 mmol/L concentration of HP-β-CD at 25 °C. The apparent stability constant (*K*c) for the complex was calculated to be 860.2 M^{-1}, which indicated a stable complex formation, since K_c in the range of 200–5000 M^{-1} suggests good complexation ability (Kane et al. 2010). This also suggests that there will be an increase in the dissolution profile which would certainly increase bioavailability of the drug.

2.3. Thermodynamics studies

The formation of host-guest complex is a dynamic equilibrium process. In this process, the effect of temperature on the complexation is an important factor. Table 1 shows the *K*c values at different temperatures. For all the temperatures examined, the *K*c values gradually reduced with the rise of temperature. The complexation ability followed the order: 298, 308, 318 K.

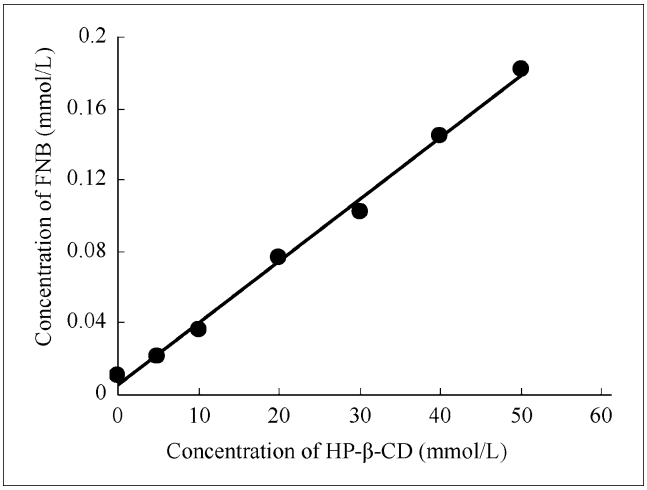

Fig. 2: Phase solubility diagram of FNB and HP-β-CD in water at 25 °C

Table 1: Apparent stability constant (*K*c) of FNB-HP-β-CD complex at different temperatures

t (°C)	*Kc*(M^{-}1)	ln*K*c	*T*(K)	1/*T* (K^{-1})
25	860.24	6.757	298	0.003354
35	581.10	6.365	308	0.003245
45	360.84	5.888	318	0.003143

This suggests that lower temperature was favorable to the formation of FNB-HP-β-CD complex, and the complex was inclined to disassociate when the temperature was raised. This was due to the fact that the movement of the included guest molecules tended to increase with increased temperature. Hence, higher temperature was unfavorable for the complex formation.

The thermodynamic parameters such as ΔG, ΔH and ΔS for the complexation process are depicted in Table 2. ΔG was negative which implies that the complexation process could proceed spontaneously at all the temperatures examined. Meantime, ΔH and ΔS were also negative which indicates that the inclusion process was an exothermic and enthalpy-driven reaction. The negative enthalpy change (ΔH) arose from the vander waals interaction, while the negative entropy change (ΔS) was due to the steric hinderance caused by molecular geometrical shape and the limit of CD cavity to the freedom of shift and rotation of guest molecules (Brewster et al. 2007). The above results indicated that the complexation process of FNB with HP-β-CD was an exothermic process accompanied by negative entropy change. In fact, the actions that enthalpy and entropy change played were on the contrary. That is to say, the ΔH was largely compensated for by the ΔS.

2.4. IR spectroscopy and X-ray diffraction studies

Fig. 3 shows IR spectra of FNB, HP-β-CD, FNB solid dispersion and the physical mixture. IR spectrum of FNB was characterized by the intense absorption of carbonyl groups, located in the bands at 1728 and 1650 cm^{-1}. Moreover, the sharp and intense absorption peak at 1600 cm^{-1} could be assigned to the benzene ring stretching vibration of the drug. The intensity of above mentioned IR absorption peaks was obviously weaker in the solid dispersion than in FNB alone or in the physical mixture. Finally, for IR spectra of the FNB solid dispersion, some absorption peaks of FNB located in other bands disappeared or their intensity became weak.

The X-ray powder diffraction patterns of the samples are shown in Fig. 4. The drug exhibited significant and sharp diffraction peaks due to its crystallinity. However, HP-β-CD showed no diffraction peaks, indicating its amorphous state. In the diffraction curves of the physical mixture, the characteristic peaks of FNB still existed. For the solid dispersion, the main diffraction peaks of the drug disappeared and the diffraction curves were similar to those of HP-β-CD, demonstrating that the samples were basically amorphous. These results in combination with IR study indicated that the FNB in the solid dispersion could exist mainly as amorphous state. Meanwhile, small amount of

Table 2: The thermodynamic parameters for the complexation process of FNB with HP-β-CD

ΔG (kJ·mol^{-1})			ΔH (kJ·mol^{-1})	ΔS (J·mol^{-1}·K^{-1})
298K	308K	318K		
−16.74	−16.30	−15.57	−34.21	−58.41

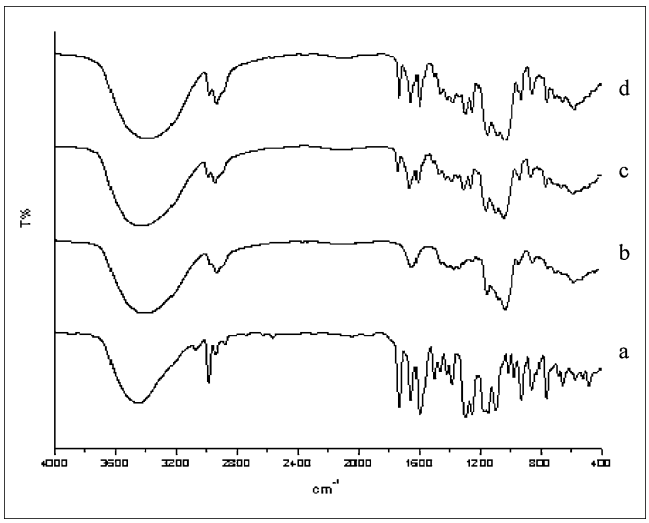

Fig. 3: IR spectra of (a) FNB, (b) HP-β-CD, (c) solid dispersion and (d) physical mixture

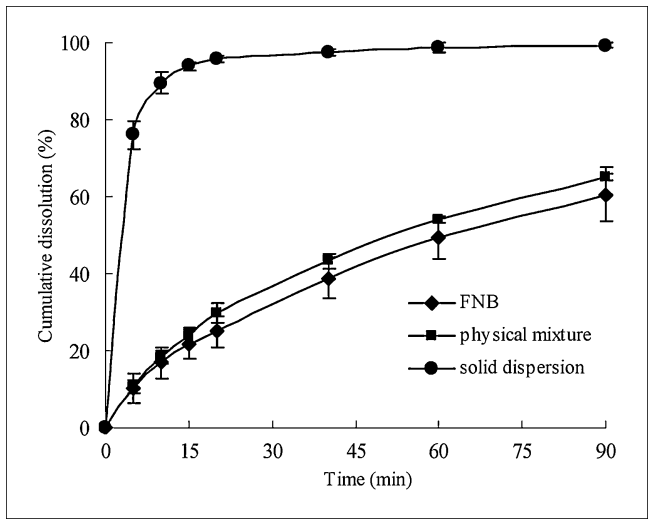

Fig. 5: Dissolution profile of FNB, physical mixture and solid dispersion (n = 3)

the drug might form the complex with HP-β-CD and exist as molecular state.

2.5. Solubility and dissolution studies

The solubility of FNB in water was found to be 3.82 mg/ml at 25 °C, however, the solubility of the drug solid dispersion was 48.63 mg/ml. That is to say, the solubility of the drug was increased by about 12-fold. As shown in Fig. 5, the FNB solid dispersion provided nearly complete drug dissolution in 40 min. However, for FNB alone or the physical mixture, only 40% or less of the drug dissolved. The results confirmed that HP-β-CD could significantly improve the solubility and dissolution behavior of the poorly water-soluble FNB. Several reasons may explain the above phenomenon. On one hand, as a solid dispersion agent, HP-β-CD could effectively reduce particle size and subsequently increase surface area of FNB, resulting in the improved dissolution rate (Sanganwar et al. 2008). On the other hand, as a CD inclusion material, HP-β-CD might form the host-guest complex with the drug, also leading to the improved wettability, the enhanced solubility and dissolution profile. Therefore, HP-β-CD possessed great advantages over the commonly used water soluble solid dispersion carrier such as PEG4000, 6000 and PVP, etc., since these carriers had no ability to include the guest molecules and subsequently improve their solubility.

According to the relevant theory of pharmacokinetics and biopharmaceutics, the dissolution of poorly water-soluble drugs in gastrointestinal tract is generally the rate-limiting step of their *in-vivo* absorption (Zhang et al. 2005), so it is reasonable to predict that the FNB-HP-β-CD solid dispersion would exhibit better bioavailability than the intact drug. Finally, since low toxic acetone was used to dissolve the drug in the preparation process of FNB-HP-β-CD system, a method to fully remove the organic solvent from the binary solid system remains to further study.

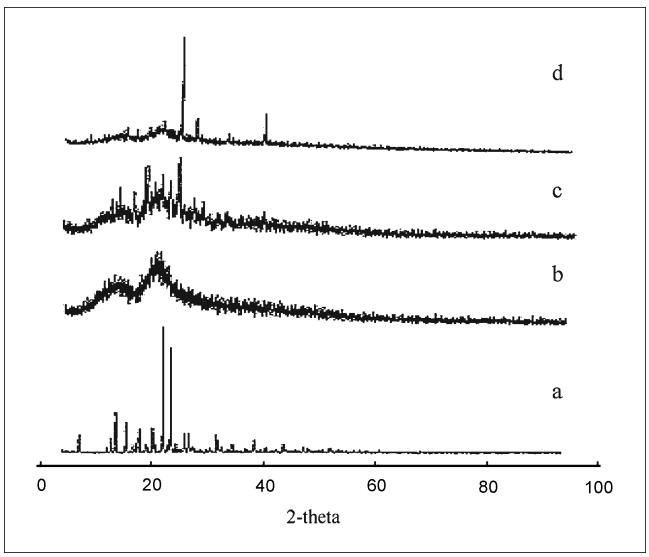

Fig. 4: X-ray diffraction patterns of (a) FNB, (b) HP-β-CD, (c) solid dispersion and (d) physical mixture

3. Experimental

3.1. UV spectroscopic studies

The complex formation of FNB with HP-β-CD was studied by the UV spectral method (Connors et al. 1966). FNB with a purity of 100.3% was kindly supplied by Wuhan Yinhe Chemical Industrial Company (Hubei, China). HP-β-CD with a purity of 99.0% and an average degree of substitution of 4.9 was purchased from Shandong Xinda Jingxi Chemical Industrial Company (Shandong, China). All other chemicals and solvents were of analytical reagent grade obtained from commercial sources and used as received without further purification. FNB stock solution of 1.1×10^{-4} mol/L was prepared by directly dissolving the drug in 30% (w/v) ethanol solution. HP-β-CD stock solution of 1.1×10^{-3} mol/L was also prepared by dissolving the adjuvant in distilled water. 1.0 ml of the FNB stock solution was first transfered to a 10 ml volumetric flask, appropriate amount of the HP-β-CD stock solution was then added. The mixture solution was diluted to a final volume of 10 ml with 30% ethanol solution. As a result, the concentration of the drug was kept constant at 1.1×10^{-5} mol/L and the concentration of HP-β-CD varied from 0 to 8.8×10^{-4} mol/L. The UV absorption spectra of the above sample solutions were recorded with a UV-2401PC spectrophotometer (Shimadzu, Japan) in range of 200–400 nm using the corresponding HP-β-CD solutions as blank.

3.2. Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (1965). An excess amount of FNB was added to 10 ml of aqueous solutions containing increasing concentrations of HP-β-CD (from 0 to 50 mmol/L). This series of suspensions were then shaken at 25 °C for 48 h, time considered enough to reach the equilibrium. All the suspensions were filtered through a 0.45 μm membrane filter and then properly diluted with water. Samples were assayed spectrophotometrically at 290 nm. The presence of trace amounts of HP-β-CD did not interfere with the assay. The apparent stability constants (*Kc*) for FNB-HP-β-CD complex was calculated from the slope and intercept of the straight portion of the phase solubility diagram.

3.3. Thermodynamics studies

Effect of temperature on the complexation process was performed at 25, 35, 45 °C, respectively, according to the above phase solubility method. The *K*c at different temperatures were then determined. Three important thermodynamic parameters were involved in the inclusion process. The ΔG could be calculated from the *K*c by the equation ($\Delta G = -RT \ln K$c). Meantime, according to the Vant Hoff equation (Li et al. 2003), ln*K*c values were plotted as a function of the inverse temperature ($1/T$) to give linear relations. Finally, the ΔH and ΔS could be obtained from the slope and the intercept of the curve.

3.4. Preparation of FNB solid dispersion

A solid dispersion of FNB with HP-β-CD in 1:1 molar ratio was prepared by the co-grinding method. Accurately weighed FNB was first dissolved in minimum volume of acetone, the resultant solution was then added dropwise and fully ground with HP-β-CD in a mortar. Acetone in the mass was finally removed by low temperature evaporation in a dryer. The obtained powder was passed through No. 80 mesh sieve for further use. A physical mixture of the drug with HP-β-CD in the same molar ratio was prepared by simply mixing the two components.

3.5. IR spectroscopy and X-ray diffraction studies

Infrared spectra of the samples were obtained using a FT-670 IR instrument (Nicolet Corporation, USA). The samples were mixed with KBr for preparing the tablets. The final spectra were performed in range of 400–4000 cm^{-1} with 2 cm^{-1} resolution. Crystallinity of the samples were analyzed using an X-ray diffractometer (Bruker Corporation, Germany) which is equipped with a Cu Kα radiation source at 40 kV voltage and 40 mA current. Diffraction patterns were obtained in 2θ range of 5–80° using 0.05° step size and 5 °/min scan speed.

3.6. Solubility and dissolution tests

Excess amounts of the drug and its solid dispersion were added to 5 ml of distilled water and then shaken at 25 °C for 48 h. The samples were finally filtered through 0.22 μm membrane filter, properly diluted and finally assayed by UV spectrometry at 290 nm.

The dissolution studies were conducted using a ZRS-8 intelligence dissolution tester (Tianjin, China) based on the Chinese Pharmacopoeia Method type II apparatus (paddle method). Approximately 25 mg of FNB, equivalent amount of the FNB solid dispersion and the physical mixture were accurately weighed and then placed in 900 ml of dissolution media thermostatically maintained at 37 ± 0.5 °C. The dissolution media was 1% sodium lauryl sulfate solution and the stirring speed was set at 100 r/min. At specific time intervals, 5 ml of the samples were withdrawn and immediately filtered through 0.45 μm filter. Meawhile, equal volume of the dissolution media were added to keep the volume constant. The filtrates were appropriately diluted prior to analysis by UV spectrometry at 290 nm. Calculation curve of absorbance versus concentration was constructed according to the solution of the drug in dissolution medium, ranging in the concentration from 2.5 to 17.5 μg/ml.There was a good linearity relationship over the concentration range. All the dissolution data were obtained in triplicate.

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