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PREPARATION AND CHARACTERIZATION OF FLORFENICOL INCLUSION COMPLEXES WITH THREE CYCLODEXTRINS

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ABSTRACT

Florfenicol inclusion complexes with three cyclodextrins were prepared for the improvement of the solubility and dissolution rate of florfenicol. The inclusion complexes were prepared by the saturated aqueous solution method with hydroxypropyl -β-cyclodextrin, γ-cyclodextrin and hydroxypropyl -γ-cyclodextrin as carriers. The products were characterized by phase solubility diagram, differential thermal analysis and dissolution rate. Stability constants for florfenicol in the three carrier solutions were calculated. The solubility of florfenicol, physical mixture and inclusion complex were measured. The results showed that the florfenicol was included by the three carriers and obeyed a typical AL-type. The stability constant was 719.73 L/mol, 352.7 L/mol and 678.91 L/mol for HP-β-CD, γ-CD and HP-γ-CD, respectively. The spectra of differential thermal analysis of the inclusion complexes were different from florfenicol and the physical mixture. Solubility of florfenicol was enhanced for the formation of inclusion complex. The dissolution rate of florfenicol inclusion complex made with HP-β-CD was higher than that of inclusion complexes made with HP-γ-CD andγ-CD. The florfenicol inclusion complexes made with three cyclodextrins showed superior performance in improving dissolution rate properties, The method was simple and practical on preparation the inclusion complexes.

KEY WORDS: Florfenicol, Hydroxypropyl-β-Cyclodextrin, γ-Cyclodextrin, Hydroxypropyl - γ-Cyclodextrin, Inclusion Complexes, Dissolution rate, Solubility.

INTRODUCTION

Florfenicol (FF, Fig. 1.) is a fluorinated synthetic analog of thiamphenicol and broad spectrum antibiotic. The chemical name is 2, 2-dichloro-N-[(*1R, 2S)*-3- fluoro-1- hydroxy -1-(4-methanesulfonylphenyl) propan- 2-yl] acetamide. FF is currently indicated for the treatment of respiratory tract infections, typhoid, intestinal infections and so on. Compared to thiamphenicol, FF shows significant superiority in antibacterial spectrum, antibacterial activity and considerably lower side effect; its antibacterial potency is 10 times higher than that of thiamphenicol [1-6]. However, due to FF relatively poor water-soluble and low dissolution in gastric fluids, the most common preparation of FF is premix formulations in market. It shows variation in bioavailability. A small number of water-soluble preparations were made by adding organic solvents, solubilizer or hydrotropy agent. But the preparations were always less stable, toxic and stimulating. It is necessary to enhance the solubility and bioavailability of FF through pharmaceutical preparation technology.

The preparation of cyclodextrin (CD) inclusion complexes is often a relatively simple and effective pharmaceutical procedure. It is widely used in the field of pharmaceutical preparation for dissolution enhancement [7-9]. Natural CD represents an important class of molecular reaction vessels that are cyclic oligosaccharides consisting of six, seven, and eight *D*-glucose units joined by *R*-1, 4- linkages for α-, β-, and γ-CD, with hydrophilic outer surfaces and a hydrophobic cavity, respectively [10]. Taking into account the advantages of the CDs in terms of good solubility in water, ready availability, inherent chirality, and transparence in UV-vis regions, CDs have the ability to encapsulate guest hydrophobic molecules of opportune polarity and dimension inside their cavities because of their special molecular structure play an important role in improving the therapeutic efficacy of drugs with poor solubility and/or stability problems. The water solubility of the guest can increase as well as its bioavailability for the inclusion complexes [11, 12].

2- hydroxypropyl -β-cyclodextrin (HP-β-CD) and 2- hydroxypropyl –γ-cyclodextrin (HP-γ-CD) were hydroxypropylated derivative of β-CD and γ-CD. The various cyclodextrin derivatives have extensively expanded the applications of these cyclic oligosaccharides and overcome the serious issues of parent CDs such as low water solubility and toxicity [13, 14]. They can form inclusion complexes with drugs of poor solubility and/or stability problems as β-CD and γ-CD [15, 16]. The aim of this study was to improve the solubility and bioavailability of FF in aqueous solution through the formation of inclusion complexes with HP-β-CD, γ-CD

and HP-γ-CD as carriers. The inclusion complex was prepared by saturated aqueous solution method. Differential thermal analysis (DTA) and dissolution rate profiles were carried out to validate the formation of inclusion complexes. The solubility of inclusion complexes were investigated and compared with those of their physical mixtures and FF alone.

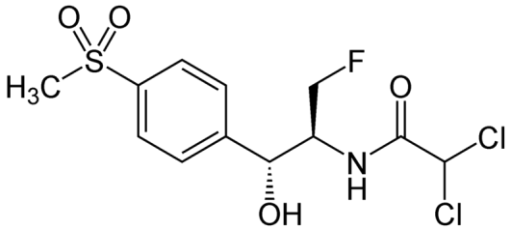

Figure (1): Chemical structure of FF

MATERIALS AND METHODS

Chemicals and Drugs

FF of 98.5% purity was received as gifts from Zhongzhou Pharmaceutical Co. Ltd (Zhengzhou, China). HP-β-CD, γ-CD and HP-γ-CD and other reagents of analytical grade were purchased from Xinshiji Chemicals Co. Ltd (Xinxiang, China) and used without further purification. The distilled water in the study was purified with Smart2 Pure 12 UV/UF purification system (Thermo Fisher Scientific, USA).

Preparation of Physical Mixtures

The individual physical mixture of FF and HP-β-CD, γ-CD and HP-γ-CD (molar ratio 1:1) was prepared by thoroughly mixing the two components with a stirrer. The mixtures were filled in glass bottles, sealed and stored in desiccators until further use.

Preparation of Inclusion Complexes

The inclusion complexes of FF with HP-β-CD, γ-CD and HP-γ-CD were prepared by saturated aqueous solution method that was edited by Lu [17]. FF, HP-β-CD, γ-CD and HP-γ-CD (molar ratio 1:1) were individually accurately weighed. FF was dissolved in an appropriate amount of ethanol to its saturation solubility and dispersed in aqueous solution of HP-β-CD, γ-CD and HP-γ-CD. After complete dissolution of FF, The mixture were stirred constantly at 50℃ in a water bath for 3h. The resultant solutions were evaporated to dryness under vacuum in a water bath at 40℃. The finally products were filled in glass bottles, sealed and stored in desiccators until further use.

Phase Solubility Diagram

Phase solubility studies were carried out in water according to the method described by Higuchi and Connors [18]. Excess amount of FF was added to 10 ml aqueous solution individually containing HP-β-CD, γ-CD and HP-γ-CD (0 - 0.01 mol/L). The suspension was mechanically shaken at 25 ± 0.5℃ for 72 h. After attainment of equilibrium, the all mixtures were centrifuged at 4000 rpm for 5 min, filtered through a 0.45 μm cellulose acetate membrane filters and suitably diluted. The absorbance of FF was recorded spectrophotometrically at a λ_{max} of 266 nm [4, 19-21]. Stability constant (*Ks*) was calculated from the slope of the phase solubility diagram using Equation 1.

$$Ks = \frac{Slope}{S_0(1 - Slope)} \quad \text{..... (1)}$$

Where S_0 is the solubility of FF in water

Saturation Solubility Studies

Saturation solubility studies were carried out in water at 25 ± 0.5℃. 200 mg FF, a quantity of FF inclusion complex and the physical mixture (molar ratio 1:1) equivalent to 200 mg of FF were sealed in glass vials with 10 mL water and stirred vigorously in a shaker water bath at 25 ± 0.5℃ for 72 h. The samples were then centrifuged and filtered through 0.45 μm cellulose acetate membrane filter. After suitable dilution, the absorbance was recorded spectrophotometrically at 266 nm.

Dissolution Rate Studies

Dissolution rate studies for FF, FF inclusion complexes and physical mixtures (molar ratio 1:1) prepared with HP-β-CD, γ-CD and HP-γ-CD were carried out in a ZRC-6FT dissolution apparatus using the first method described in ChP2010. The temperature was set at 37 ℃ and the paddle rotated at 100 rpm. All of the samples of 100 mg FF or its equivalent for the physical mixture or inclusion complexes were added to 1000 ml distilled water. 5mL solution was withdrawn at 2.0, 5.0, 10.0, 15.0, 20.0, 30.0 and 45.0 min, respectively. All the solutions were immediately filtered (0.45 μm pore filter), suitably diluted, and absorbance recorded spectrophotometrically at 266 nm. Equivalent volume of fresh water pre-warmed to 37℃ was used to replenish the medium after each sampling. The cumulative percentage of FF dissolved was calculated from the regression equation generated from the standard data.

Differential Thermal Analysis (DTA)

DTA thermograms of FF, three cyclodextrins, FF inclusion complexes and physical mixtures (molar ratio 1:1) prepared with HP-β-CD, γ-CD and HP-γ-CD were carried out with a CRY-32P DTA instrument. Each sample (5 mg, accurately weighed) was heated in an aluminum pan at a rate of 10℃/min from 30 to 300℃ under air flow. The thermograms were compared with one another regarding in terms of peak position, peak shift, and presence/absence of peaks at particular temperatures.

RESULTS AND DISCUSSION

FF inclusion complexes with improved solubility using HP-β-CD, γ-CD and HP-γ-CD as carriers were formed by saturated aqueous solution method. It is a basic pharmaceutical procedure used to prepare inclusion complexes. The method is simple, easy, and less expensive for preparation of inclusion complexes to carry out on a laboratory or industrial level.

Phase Solubility Diagram

Phase solubility studies of FF in three different carriers solutions were carried out. The results were shown in Fig. 2 a-2c. The diagrams show that the aqueous solubility of FF increased in a linear manner as a function of HP-β-CD, γ-CD and HP-γ-CD concentration, which resulted in A_L-type phase solubility diagram based on the Higuchi and Connors model. The stability constants were 719.73 L/mol, 352.7 L/mol and 678.91 L/mol for HP-β-CD, γ-CD and HP-γ-CD, respectively. Typical regression equations were calculated as follows: y = 0.6972x + 0.0032, y = 0.4969x + 0.0028 and y = 0.6707x + 0.003 for HP-β-CD, γ-CD and HP-γ-CD, where y is the concentration (mol/L) of FF and x is the concentration (mol/L) of the three different carriers.

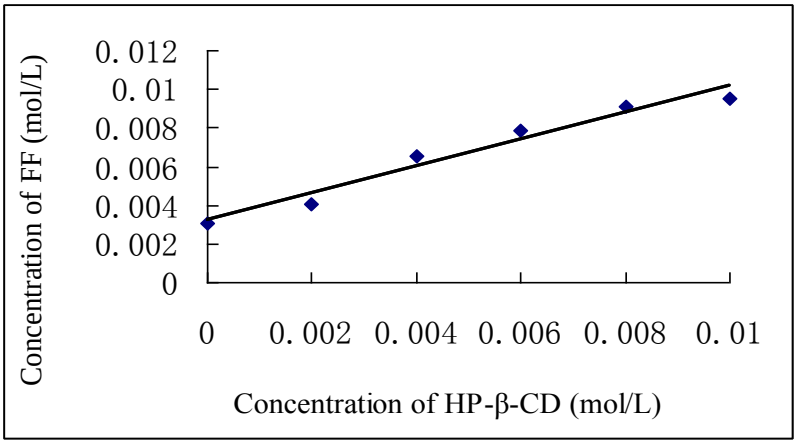

Figure (2a): Phase solubility diagram of FF/ HP-β-CD system in water

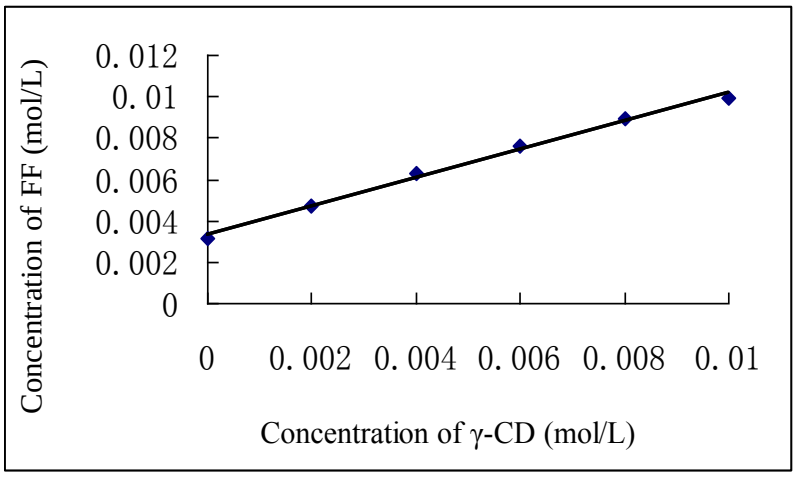

Figure (2b): Phase solubility diagram of FF/γ-CD system in water

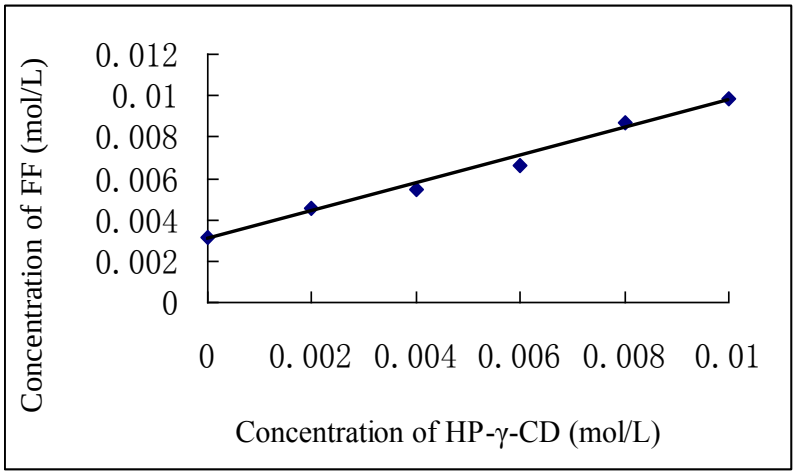

Figure (2c): Phase solubility diagram of FF/ HP-γ-CD system in water

Saturation Solubility Studies

The saturation solubility of FF at room temperature was 1.118 mg/ml. The saturation solubility of FF inclusion complexes and the physical mixtures prepared with HP-β-CD, γ-CD and HP-γ-CD were shown in Table 1.

Table (1): Solubility Studies Results of FF Inclusion Complexes and Physical Mixtures.

Excipients	Inclusion complexes (mg/ml)	Physical mixtures (mg/ml)
HP-β-CD	10.686	3.029
γ-CD	8.873	2.326
HP-γ-CD	9.467	2.922

Dissolution Rate Studies

Dissolution rate studies for FF, FF inclusion complexes and physical mixtures were carried out. The dissolution profiles of all samples were shown in Fig. 3 a-3c.

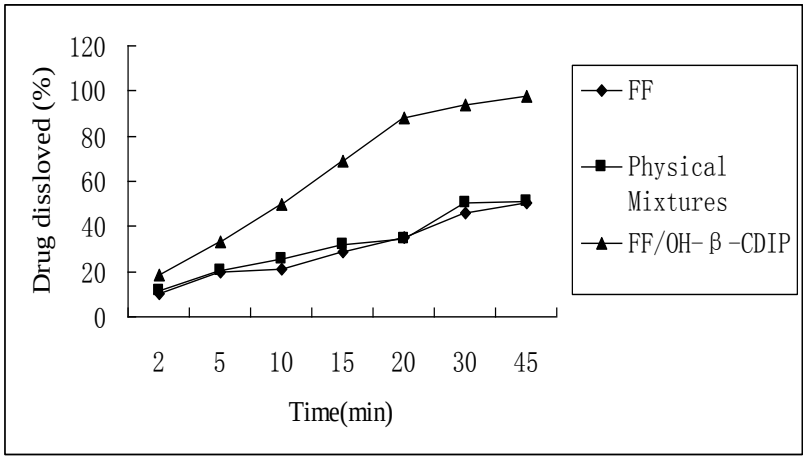

Figure (3a): Dissolution Rate Profile of FF, Physical Mixtures And FF/HP-B-CD Inclusion Complex.

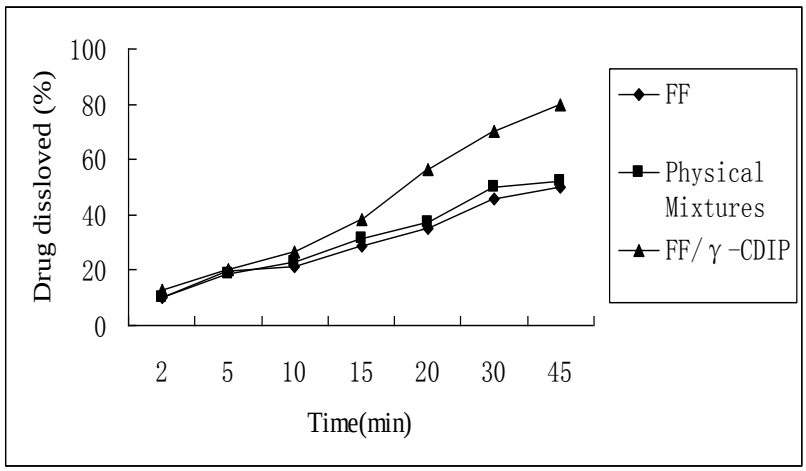

Figure (3b): Dissolution Rate Profile of FF, Physical Mixtures And FF/Γ-CD Inclusion Complex.

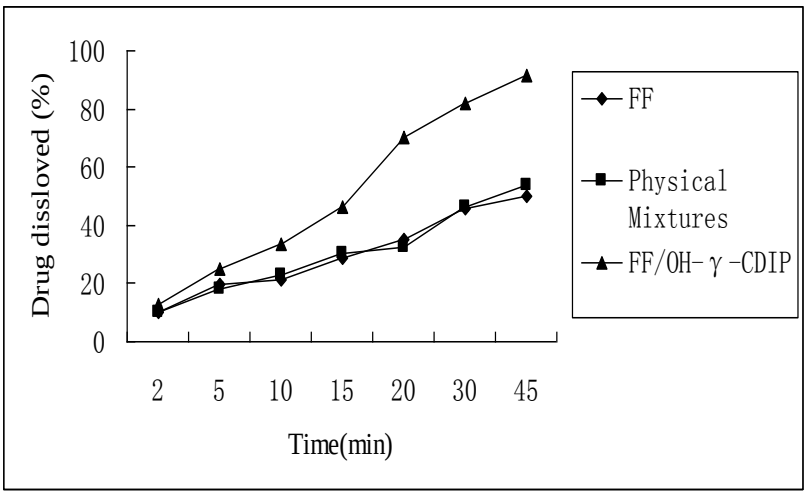

Figure (3c): Dissolution Rate Profile of FF, Physical Mixtures and FF/ HP-Γ-CD Inclusion Complex.

The three inclusion complexes evidently exhibited increased dissolution rate than FF and physical mixtures. For the FF/HP-β-CD and FF/ HP-γ-CD inclusion complex, the amount dissolved after 45 min was more than 92%, thus, the two inclusion complex were effective in improving the drug dissolution behavior. The dissolution rate of FF/HP-β-CD inclusion complex was higher than that of inclusion complex made with HP-γ-CD and γ-CD. The key reason for the superiority of HP-β-CD is probably the result of greater water solubility, higher wetting, appropriate diameter of host molecular, hydrophilic groups and complexation to FF. The main conditions for the formation of FF inclusion complex are the three- dimensional structure and polarity of FF and carriers. The stability of the inclusion complex formed by the two molecules depends on the strength of the van der Waals force, dispersion force, hydrogen bonding, charge transfer, etc. It is often the result of a single force or the synergy of several forces. The size and shape of the FF should be adaptive with carriers; otherwise it would have been difficult to form a stable inclusion complex.

Differential Thermal Analysis (DTA)

DTA provided the evidence that inclusion complexes were formed and performed. When FF was imbedded in CD cavities or crystal lattice, it's melting, boiling, or sublimation point generally shifted to a different temperature or disappears within the temperature range where excipients decompose. The DTA thermograms of FF, three cyclodextrins, physical mixture and inclusion complexes (molar ratio 1:1) prepared with HP-β-CD, γ-CD and HP-γ-CD were shown in Fig. 4 a-4c.

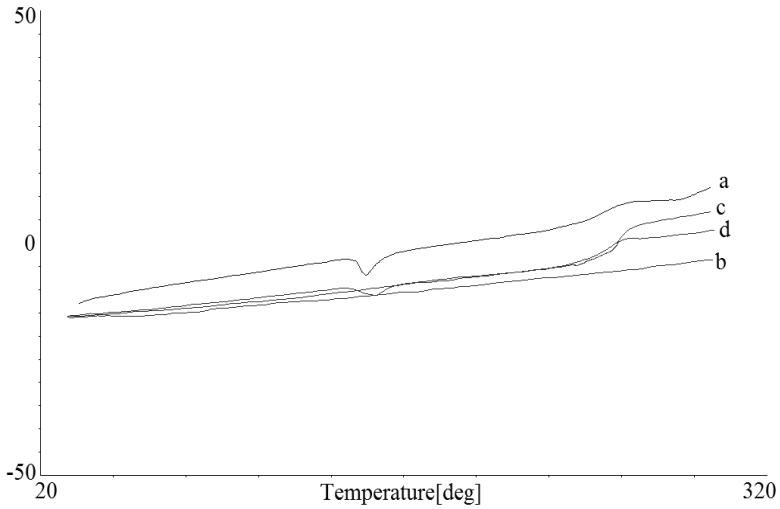

Figure (4a): DTA Thermograms of FF (A), HP-B-CD (B), Physical Mixture(C) and Inclusion Complexes (D).

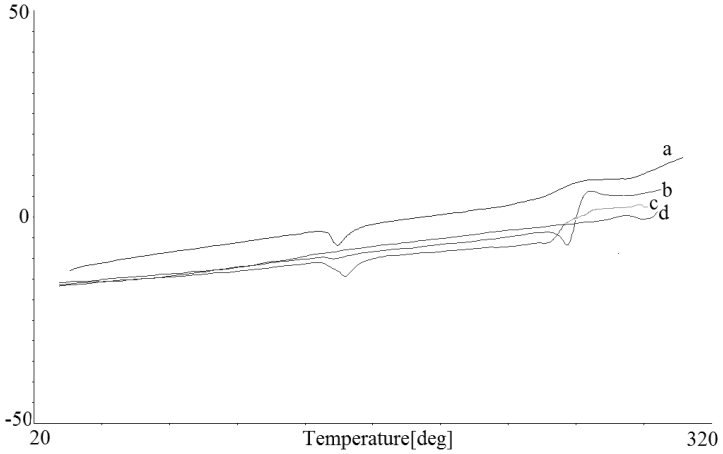

Figure (4b): DTA Thermograms of FF (A), Γ-CD (B), Physical Mixture (C) And Inclusion Complexes (D).

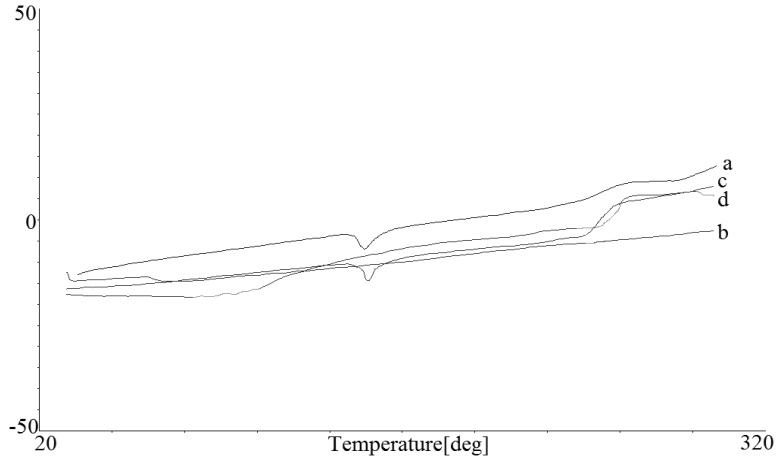

Figure (4c): DTA Thermograms of FF (A), HP-Γ-CD (B) Physical Mixture (C), And Inclusion Complexes (D)

The melting point of FF was at 154.2 °C exhibiting with an endothermic peak. The thermal behavior of HP-β-CD and HP-γ-CD did not show any obvious absorption peak, but rather an oblique line. The DTA thermogram of γ-CD exhibited an endothermal peak at 267°C indicating its melting point. In the thermogram of the inclusion complex, the endothermal peak of FF was not observable, indicating physical interaction of FF with HP-β-CD, HP-γ-CD and γ-CD and the amorphous characteristic of the samples, resulting in an almost complete loss of crystal form of the binary system. In contrast, the physical mixture showed a clearly visible endothermal peak for FF, the thermograms of the physical mixtures were similar to the superimposition of thermograms of individual FF and the carriers. The DTA results indicate complete formation of inclusion complex at 1:1 molar ratio, and this is consistent with the phase solubility data obtained.

CONCLUSION

In this study, the results show that HP-β-CD, HP-γ-CD and γ-CD can be used as carriers in FF inclusion complexes and that the saturated aqueous solution method is a promising approach for achieving higher drug solubility. The inclusion complexes were characterized by phase solubility diagram, DTA and dissolution rate. All the inclusion complexes shown improved dissolution rate in comparison with starting material and physical mixtures. The inclusion complexes result in improved drug solubility and dissolution rate.

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