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Binary and ternary inclusion complexes of finasteride in HPβCD and polymers: Preparation and characterization

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ABSTRACT

The aim of this study was to determine whether inclusion complexes between 2-hydroxypropyl-β-cyclodextrin (HPβCD) and finasteride (FIN) are formed, and to characterize these. Equimolar FIN/HPβCD solid systems in the presence or absence of 0.1% (w/v) of polyvinylpyrrolidone K30 (PVP K30) or 0.3% of chitosan were prepared by coevaporation and freeze-drying methods. The systems were characterized by phase solubility, NMR, DSC, and XRD analysis. The results suggest that true binary and ternary inclusion complexes were formed.

1. Introduction

Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity that can accommodate a variety of lipophilic drugs.[1] The formation of complexes usually results in favorable changes in many of the physicochemical properties of the drug, such as solubility, dissolution rate, stability, and bioavailability. It should be stressed, however, that pharmaceutical dosage forms should contain as little CD as possible, because excess CD can cause some problems in formulation bulk or potential toxicity, as well as reducing bioavailability and preservative efficacy.[2] Therefore, in cases where low complexation efficiency would require a larger amount of CD than that acceptable for solid or liquid dosage forms, enhancement of the complexation capacity of the chosen CD is of practical importance. Many authors have reported the positive effect of the addition of small amounts of polymers to a drug-CD system in order to improve its complexation and solubilization efficiencies.[2–4]

Finasteride (FIN, Fig. 1) is an orally administered 5-alpha-reductase inhibitor that blocks the conversion of testosterone to dihydrotestosterone.[5] The goal of therapy with FIN is to reduce prostate volume, increase urinary flow, improve symptoms, and prevent the progression of benign prostatic hyperplasia.[6] It is a steroidal molecule with a molecular weight of 373 Da[7,8] and is soluble in polar organic solvents such as ethanol, methanol, and chloroform, and practically insoluble in water. FIN is rapidly absorbed from the gastrointestinal tract, and is observed to have a bioavailability of 63–80%.[5,9]

The effect of CDs and polymers on the solubility of FIN was studied by Loftsson et al.,[10,11] who investigated ways of improving the solubility of this drug. The present study, however, investigated the effect of a CD and polymers on the solubility of this drug in the solid state. The objective of this work was to study the interactions of FIN with 2-hydroxypropyl-β-cyclodextrin (HPβCD), with or without the presence of the natural polymer chitosan or the synthetic polymer polyvinylpyrrolidone (PVP K30). In order to evaluate the influence of HPβCD and these polymers on FIN solubility, the interactions in aqueous solution were investigated by phase solubility analysis. Therefore, the influence of HPβCD and the poly-

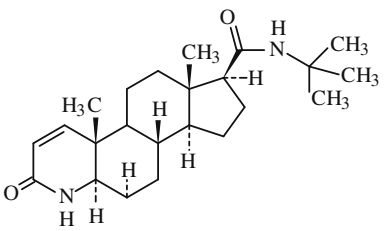

Figure 1. Chemical structure of finasteride.

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mers on the physicochemical properties of FIN was evaluated by preparing solid systems with equimolar quantities of HPβCD and FIN in the presence or absence of 0.3% chitosan or 0.1% PVP K30 by coevaporation and freeze-drying. Nuclear magnetic resonance (NMR),[12] differential scanning calorimetry (DSC), and powder X-ray diffractometry (XRD) analyses were used to characterize the binary and ternary systems and compare them with the physical mixtures prepared in the same molar ratio. Tablets were prepared with the systems and microcrystalline cellulose, and submitted to a dissolution test.

2. Results and discussion

2.1. Phase solubility studies

Polymers are known to interact with the outer surface of CDs and with drug-CD complexes, forming co-complexes or aggregates that show higher stability constants (K_c) values than those for the binary drug-CD system.[4] They increase the complexation efficiency, and therefore a smaller amount of CD can be used in the preparation of the complex.[13] The addition to the system of 0.3% chitosan or 0.1% PVP K-30 resulted in an increase of 0.73% and 7.97%, respectively, in the solubility of FIN. Figure 2 shows the effect of increasing concentrations of HPβCD, in the presence or absence of chitosan or PVP K30, on the solubility of FIN in aqueous solution. The phase solubility diagrams were all Higuchi A_L type, that is, they were characterized by a linear increase in drug solubility as a function of HPβCD concentration, indicating the formation of a first-order complex with respect to HPβCD (Fig. 2). As the slopes of all the phase solubility diagrams were less than 1, a 1:1 stoichiometry can be assumed.[14] FIN solubility increased by 56.6% in the binary system and by 64.9% and 66.6% in the ternary systems with chitosan and PVP K30, respectively (Fig. 2). The estimated values of S_0, the slopes of the phase solubility diagrams, and the K_c are shown in Table 1.

According to Rama et al.,[15] only complexes with a K_c between 100 and 1000 mol L^{-1} have industrial applications. Complexes with a lower K_c than 100 mol L^{-1} represent unstable drug-CD systems, whereas complexes with a K_c higher than 1000 mol L^{-1} could adversely affect drug absorption. The K_c values found for all the systems studied (673 for the FIN/HPβCD binary system and 641 and 616 for the FIN/HPβCD/chitosan and FIN/HPβCD/PVP K30 ternary systems, respectively) indicate that inclusion complexes with suitable stability were formed. Loftsson et al.[11] proposed another method to evaluate the solubilizing effects of CDs, which consist of determining the complexation efficiency (CE). Based on this value, the drug:CD ratio can be determined, as well as the increase in the formulation bulk in a solid dosage form. The drug-to-CD ratio, CE values, and formulation bulk are shown in Table 2.

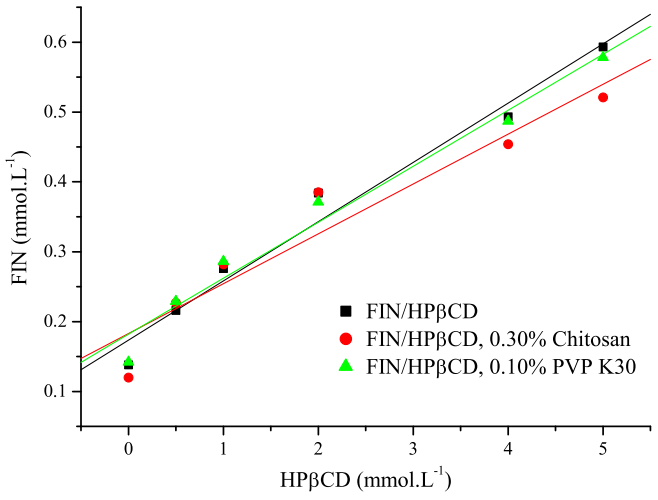

Figure 2. Phase solubility diagrams for FIN at room temperature in the presence of HPβCD without polymers, and with 0.1% (w/v) of PVP K30 or 0.3% (w/v) chitosan. The lines represent the best-fit linear regression of data points.

Table 1
Finasteride solubility (S_0), slope, stability constant (K_c), and correlation coefficient (R) from phase solubility diagrams

System	S_0 (mmol L^{-1})	Slope	R	K_c (mol L^{-1})
FIN/HPβCD	0.138	0.08482	0.9875	672.980
FIN/HPβCD/Chitosan	0.120	0.07139	0.9595	640.563
FIN/HPβCD/PVP K30	0.142	0.08017	0.9876	615.872

Table 2
Complexation efficiency (CE), molar ratio, and formulation bulk for binary and ternary systems

System	CE	Molar ratio (FIN:CD)	Formulation bulk (mg)
FIN/HPβCD	0.093	1:12	226
FIN/HPβCD/Chitosan	0.077	1:14	263
FIN/HPβCD/PVP K30	0.087	1:13	244

Loftsson et al.[10] studied the complexation of FIN with HPβCD, SBEβCD, and RMβCD. They reported CE values of 0.625, 0.678, and 0.708, respectively, and a drug:CD molar ratio of 1:3, indicating that approximately one of every three CD molecules in solution forms a water-soluble complex with FIN. Instead of only stirring systems at room temperature, they briefly heated them in an autoclave and then cooled them to room temperature. However, according to Ribeiro et al.,[4] autoclaving could produce conformational and flexibility changes in the CD cavity, which could enhance the interaction between the CD and a poorly soluble drug. The use of different methodologies in phase solubility studies could explain the differences between the values for CE and molar ratio found by Loftsson et al.[10] and those presented in this study.

The addition of chitosan or PVP K30 did not greatly affect the slope or the values of K_c or CE or the molar ratio (Tables 1 and 2). Similar results were obtained by Loftsson et al.[11] in a previous study that investigated the interaction between FIN and RMβCD in the presence of HPMC, CMC, and PVP K30. Although the formulation bulk of binary and ternary systems (about 240 mg) is much greater than the dosage of FIN (5 mg), the high potency of the drug allows the complexes to be used in solid dosage forms.

2.2. NMR analysis

^{1}H and 1D and 2D ROESY NMR experiments were performed to confirm that the FIN/HPβCD inclusion complex was formed, as well as to characterize the binding mode. The formation of a FIN/HPβCD complex was first evidenced by comparing the FIN and FIN/HPβCD ^{1}H NMR spectra under the same experimental conditions. Variations in the ^{1}H NMR chemical shifts and broadening of the signal of the olefinic hydrogens in FIN (doublets at 5.74 and 6.93 ppm) as well as those from the methyl group closest to the olefin (singlet at 0.96 ppm) were observed (Fig. 3), revealing a significant interaction between HPβCD and the first ring of the FIN structure, which contains a conjugated carbonyl (Fig. 1). This specific interaction was supported by one- and two-dimensional rotating frame nuclear Overhauser effect (1D and 2D ROESY) NMR experiments, which are usually suitable to measure nOe in complex systems. The 2D ROESY experiment showed correlations of the same FIN hydrogens referred to above, with the internal H-3 and H-5 hydrogens in the

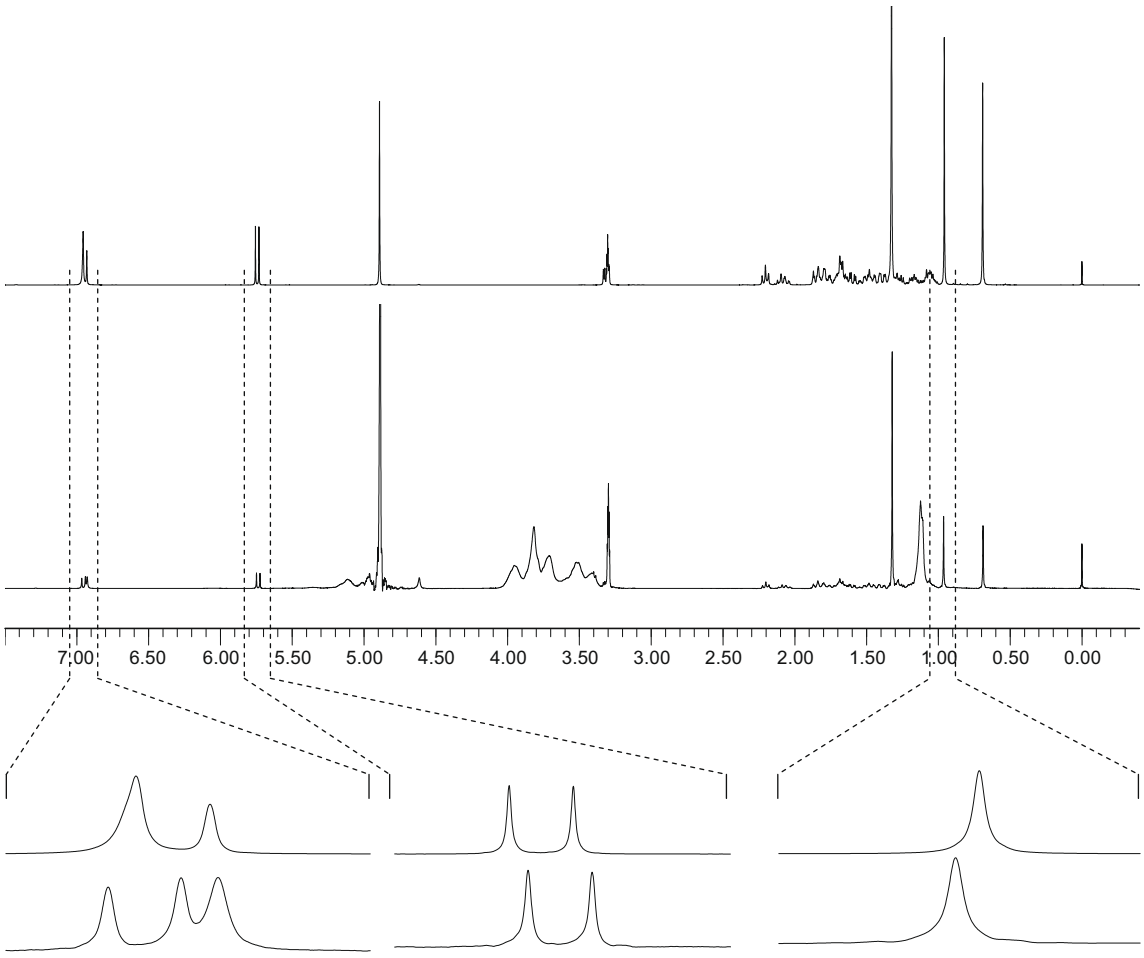

Figure 3. ^{1}H NMR spectra for FIN (top) and FIN/HPβCD complex (bottom), showing the variations in the chemical shift in the expansions.

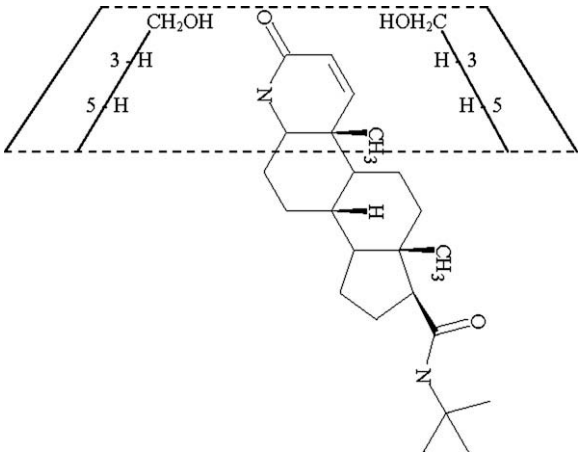

Figure 4. Representation of the FIN/HPβCD inclusion complex, showing the FIN structure partially inserted in the HPβCD cavity.

HPβCD structure (Fig. 4). This was confirmed in the 1D ROESY NMR experiment performed by selective excitation of H-3 and H-5 HPβCD hydrogens at 3.88 ppm, which caused nOe enhancement in only the olefinic hydrogens (doublets at 5.74 and 6.93 ppm) and those from the methyl group (singlet at 0.96 ppm; Fig. 5). These results revealed the formation of a specific FIN/HPβCD inclusion complex, in which only part of the FIN structure is included in the HPβCD cavity (Fig.4).

2.3. DSC analysis

DSC was used to characterize FIN complexes in the solid state, and to obtain further supporting evidence of complex formation. Whereas the thermal curve of pure FIN was typical of a crystalline anhydrous substance, with a sharp endothermic peak at 259.14 °C corresponding to the melting point of the drug, a broader endothermal peak associated with water loss was recorded for HPβCD (Fig. 6). In the FIN/HPβCD physical mixture (PM), the characteristic thermal profile of FIN was shifted to lower temperatures in the region of 163.7 °C. In the binary system, the endothermic event associated with fusion of FIN was totally absent, indicating the formation of amorphous entities and/or inclusion complexes. Comparison of DSC curves from coevaporated (COE) and lyophilized (LPh) binary systems did not show any significant differences. Similarly, comparison of DSC curves from binary systems with those belonging to ternary systems failed to reveal any significant differences. A broader endothermal peak was also recorded for PVP K30 and chitosan as a consequence of water loss. A peak that was characteristic of FIN (corresponding to melting of the drug) was clearly distinguishable in both binary and ternary PMs. In PM, the characteristic thermal profile of FIN was shifted to lower temperatures in the region of 184.5 °C for the ternary system with chitosan and 188.9 °C for the ternary system with PVP K30 (Fig. 6). The disappearance of the FIN endothermic peak in all the ternary systems obtained by coevaporation and lyophilization may be a strong indication that amorphous entities and/or inclusion complexes were formed.

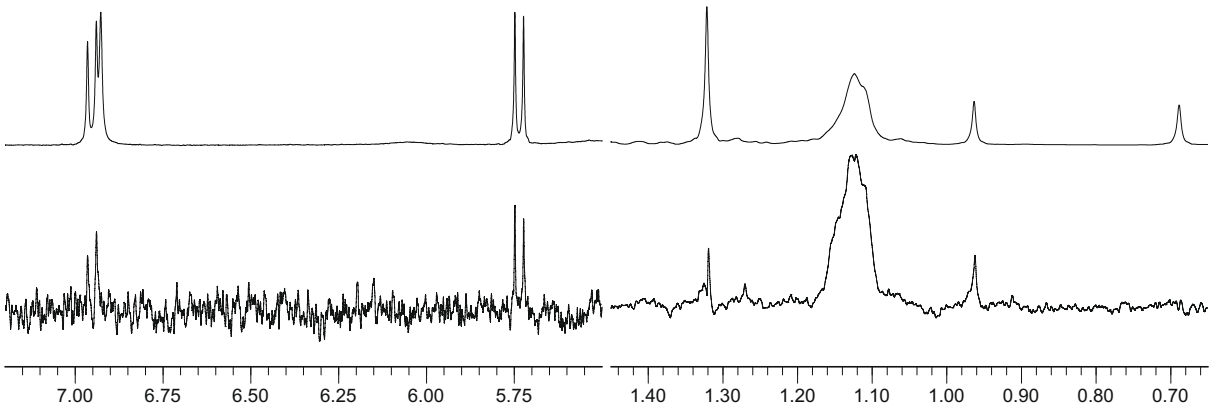

Figure 5. 1D ROESY spectrum for the FIN/HPβCD complex (bottom) obtained by selective irradiation of the signal at 3.88 ppm corresponding to H-3 and H-5 hydrogens of HPβCD, showing nOe enhancements of the FIN olefinic and methyl hydrogen signals. The top spectrum is a normal ^{1}H NMR spectrum of the complex used for comparison.

2.4. XRD analysis

Powder XRD is a useful method for detecting CD complexation in powder or microcrystalline states. The diffraction pattern of the complex should be clearly distinct from those obtained by the superposition of each component, if a true inclusion complex exists.[4,16]

The XRD pattern of FIN revealed high-intensity reflections corresponding to the diffraction peaks at 14.11°, 15.82°, and 19.23° (2θ), which were indicative of its crystalline character; whereas a hollow pattern was recorded for HPβCD, chitosan, and PVP K30, indicating their amorphous state. Some diffraction peaks attributable to FIN crystals were still detectable in the PM, whereas they were absent in the respective coevaporated (COE) and lyophilized (LPh) binary and ternary systems, indicating the formation of a true inclusion complex (Fig. 7).

2.5. Dissolution rate studies

Figure 8 shows the amount of FIN that dissolved on its own and in binary and ternary systems. All the systems exhibited a faster dissolution rate than the free drug, and the increase in the dissolution rate of FIN was twice as great when in the inclusion complex form, with the exception of binary systems obtained by coevaporation. The increase in dissolution rate in lyophilized binary and ternary complexes may be due to the high-energy amorphous state of lyophilized products.[17]

3. Conclusions

The results of this study showed that FIN/HPβCD binary and FIN/HPβCD/chitosan and FIN/HPβCD/PVP K30 ternary complexes can be formed in solution and in solid state, and also suggest that both coevaporation and lyophilization methods result in a reasonable degree of amorphization. 1D and 2D ROESY NMR experiments showed that the inclusion complex between FIN and HPβCD is formed by the insertion of the FIN ring that contains the conjugated carbonyl group in the HPβCD cavity. Neither the preparation method nor the type of the polymers used to obtain the ternary systems exerted a significant influence on the physicochemical characteristics of the products. Values of K_c and CE decreased with the addition of chitosan and PVP K30, showing that they do not have a positive effect on FIN solubility. However, the increase in the dissolution rate of the drug as well as a convenient increase in formulation bulk justify the use of binary and ternary FIN/HPβCD complexes in solid dosage forms.

4. Experimental

4.1. Materials

Finasteride (*N*-(2-methyl-2-propyl)-oxo-4aza-5α-androst-1-ene17B carboxamide) was obtained from Natural Pharma (São Paulo, Brazil); 2-hydroxypropyl-β-cyclodextrin (HPβCD) with an average degree of molar substitution of 0.6 (average molecular weight of 1400 Da) was purchased from Chemyunion (São Paulo, Brazil); polyvinylpyrrolidone K30 (PVP K30) was purchased from Basf Company (São Paulo, Brazil); and chitosan was obtained from Galena (São Paulo, Brazil). All other materials were of analytical reagent grade.

4.2. Phase solubility studies

The phase solubility assays were carried out initially with an excess amount (10 mg) of FIN added to aqueous solutions containing increasing concentrations of HPβCD from 0 to 5 mmol L^{-1} (FIN/HPβCD). Subsequently, the same initial system was performed with the addition of 0.3% (w/v) of chitosan (FIN/HPβCD/Chitosan) or 0.1% (w/v) of PVP K30 (FIN/HPβCD/PVP K30) (Table 3). The suspensions were equilibrated at room temperature under mechanical stirring for 72 h. They were then filtered (0.45 μm pore size) and analyzed by HPLC, using an Agilent® HP 1100 series (Hewlett–Packard, Waldbronn, Germany) equipped with a Luna® C-18 reversed-phase chromatographic column (150 × 4.6 mm; 5 μm particle size) conditioned inside a column oven (G1316a), a degasser (G1322A), a quaternary pump (G1311A), an automatic sampler (G1329Aa), and a UV–vis DAD detector (G1315B). The column was kept at 45 °C throughout the elution process, which used a mobile phase consisting of *o*-phosphoric acid (2.5 mM) and acetonitrile 1:1 (v/v) at a flow rate of 1.5 mL min^{-1} and the detection wavelength set to 240 nm. The presence of HPβCD and/or polymers did not interfere with the method used to analyze FIN. The apparent stability constants (K_c) of the FIN/HPβCD complex were calculated from the slope of the phase solubility diagrams and the solubility of the drug in water (S_0).[18]

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

4.3. Solid systems preparation

4.3.1. Binary and ternary physical mixtures

Equimolar physical mixtures of FIN and HPβCD were prepared by blending in a mortar until a homogeneous mixture was ob-

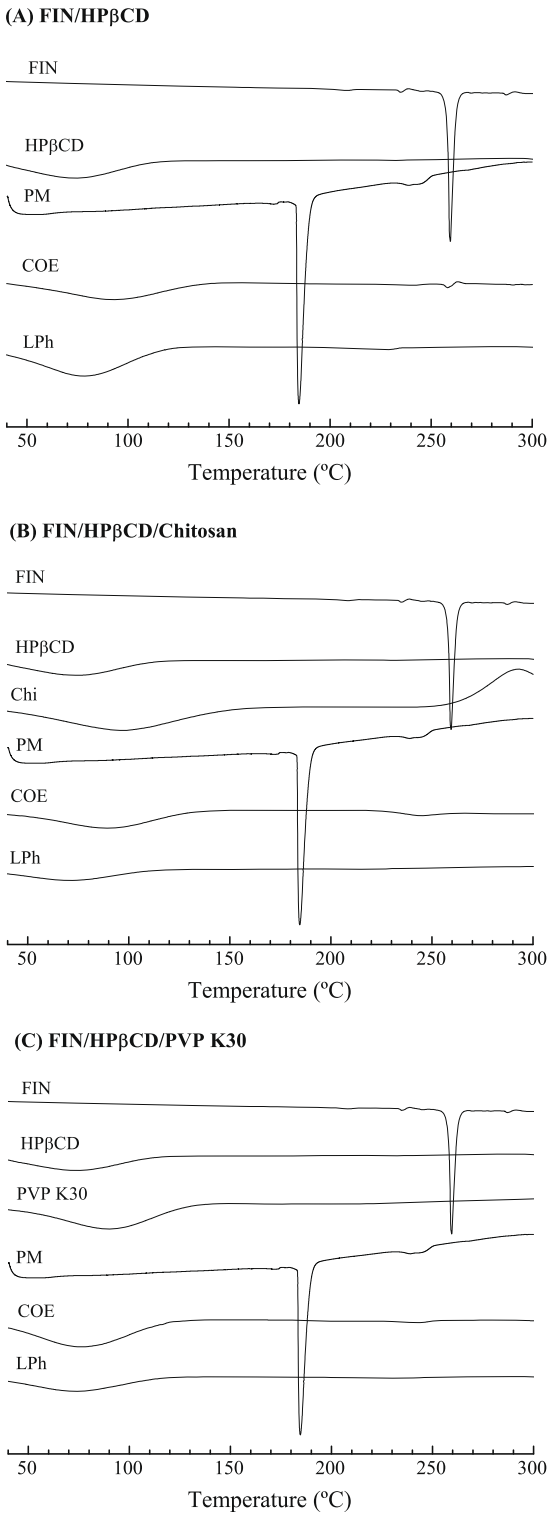

Figure 6. DSC curves of FIN, HPβCD, polymers PVP K30 and chitosan (chi), physical mixtures (PM), and coevaporated (COE) and lyophilized (LPh) binary and ternary systems obtained in dynamic nitrogen atmosphere (100 mL min^{-1}) and a heating rate of 10 °C min^{-1}.

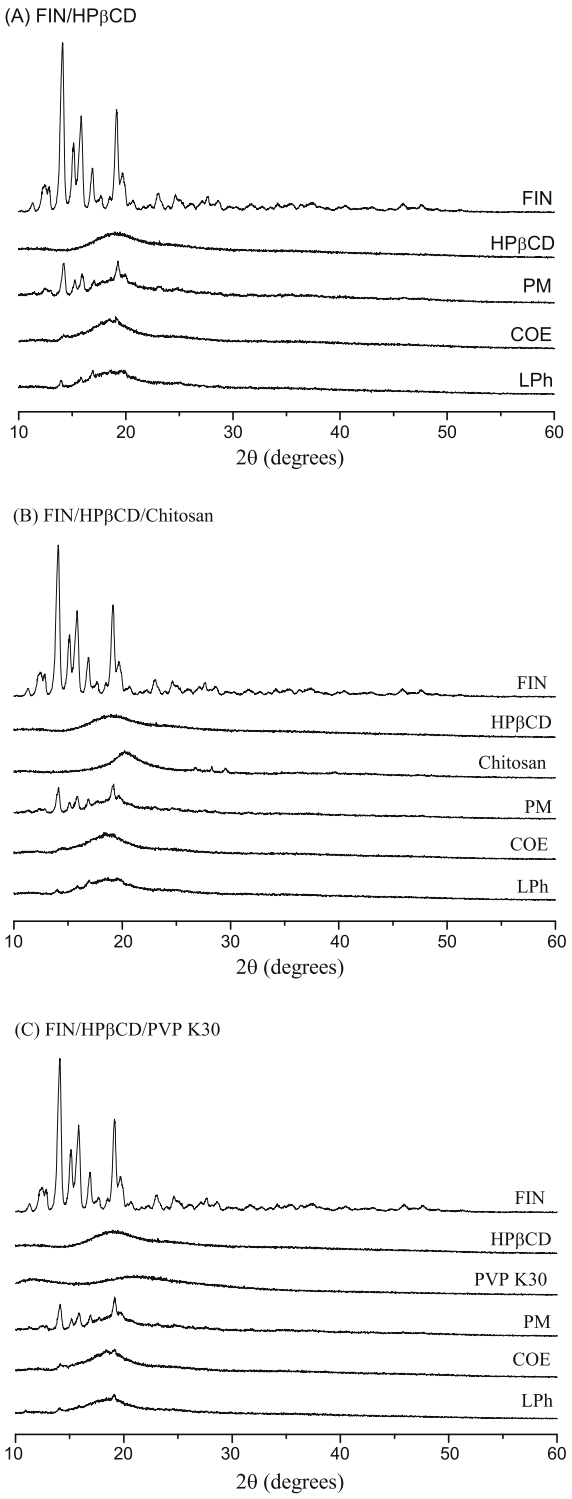

Figure 7. Powder XRD patterns of FIN, HPβCD, polymers PVP K30 and chitosan (chi), physical mixtures (PM), and coevaporated (COE) and lyophilized (LPh) binary and ternary systems.

tained. For ternary systems, PVP K30 or chitosan was added to a final concentration of 0.1% and 0.3%, respectively.

4.3.2. Coevaporated and lyophilized binary and ternary systems

Equimolar amounts of FIN and HPβCD were dissolved in ethanol and water, respectively. The two solutions were mixed and stirred magnetically for 8 h at room temperature. For coevaporation, the resulting suspension was evaporated in an oven (Tecnal® TE 394/1) at 40 °C, while for lyophilization, the suspension was freeze-dried in an Edwards® freeze dryer. The ternary systems were prepared in the same way, but chitosan or PVP K30 was added in the HPβCD aqueous solution to the same final concentrations as for the physical mixtures.

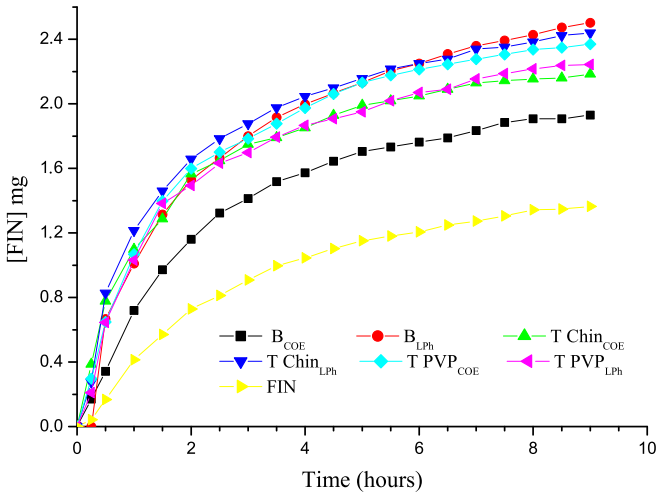

Figure 8. Amount of FIN dissolved on its own and in coevaporated and lyophilized binary (B_{COE} and B_{LPh}) and in ternary systems with chitosan (T $Chin_{COE}$ and T $Chin_{LPh}$) or PVP K30 (T PVP_{COE} and T PVP_{LPh}).

Table 3
Phase solubility formulations studied

System	FIN (mg)	HPβCD (mmol L^{-1})	PVP K30 (w/v)	Chitosan (w/v)
FIN/HPβCD	10	0–5	—	—
FIN/HPβCD/Chitosan	10	0–5	—	0.3%
FIN/HPβCD/PVP K30	10	0–5	0.1%	—

4.4. NMR analysis

The formation of a complex between FIN and HPβCD was investigated by means of NMR spectroscopy analysis. For this, ^{1}H as well as 1D and 2D ROESY NMR experiments were performed at 20 °C in MeOD-d_4 on a Bruker AVANCE 400 NMR spectrometer operating at 9.4 T, observing ^{1}H at 400.13 MHz under the lyophilized FIN/HPβCD system. The spectrometer was equipped with a 5 mm multinuclear direct detection probe with *z*-gradient. The ^{1}H NMR spectra were acquired with a spectral width of 3306.88 Hz (≅8.3 ppm) and 64 K data points, providing a digital resolution of 0.05 Hz. The 1D ROESY experiments were obtained by selective 180° pulse excitation and selective refocusing with shaped pulses using the gradient *selroegp* pulse sequence, with a mixing time of 200 or 300 ms for ROESY spin-lock, recycle delay of 1.0 s, and 512 transients. The spectra were acquired with the same spectral conditions as ^{1}H NMR. The ^{1}H and 1D ROESY NMR spectra were processed by applying a Fourier transform with zero-filling to 64 K data points and by an exponential multiplication of the FIDs by a factor of 0.3 and 1 Hz for ^{1}H and 1D ROESY NMR, respectively. The 2D ROESY NMR experiments were acquired using the gradient *roesyetgp* pulse sequence with a spin-lock of 200 or 300 ms, a spectral width of ≅8.4 ppm in f_2 and f_1, 4 K data points and 64 transients in t_2 for each of 256 increments in t_1. The relaxation delays were set to 1 s and the experiments were processed by Fourier transform using squared sine apodization in both dimensions and zero-filled to 4 K × 2 K data points in f_2 and f_1, respectively. All ^{1}H NMR chemical shifts are given in ppm in relation to the TMS signal at 0.00 ppm (internal reference), and all pulse programs were supplied by Bruker BioSpin.

4.5. DSC analysis

Samples of FIN, physical mixtures, excipients, and systems in powdered form were examined by conventional differential scanning calorimetry (DSC-910, TA Instruments Inc., USA) using aluminum crucibles with approximately 2 mg of samples in a dynamic nitrogen atmosphere (100 mL min^{-1}) and a heating rate of 10 °C min^{-1} in the temperature range of 40–300 °C. The DSC cell was calibrated with indium (mp 156.6 °C; $\Delta H_{fus.}$ = 28.54 J g^{-1}) and zinc (mp 419.6 °C).

4.6. XRD analysis

X-ray powder diffraction patterns were collected using a Shimadzu XRD-6000 powder diffractometer at room temperature. The diffractograms were recorded in the 2θ angle range between 5° and 60°, and the process parameters were set at a scan step size of 0.025 (2θ), a scan step time of 1.25 s, and an acquisition time of 1 h.

4.7. Dissolution rate studies

Dissolution studies were performed according to the USP 30 method for FIN tablets.[7] The dissolution rates of FIN and solid systems were measured in an Erweka Dissolution Tester, with the apparatus 2 in 900 mL of degassed water kept at a thermostatically controlled temperature of 37 °C ± 0.5 °C and stirred at 50 rpm. At fixed time intervals, samples were collected, filtered (0.45 μm pore size), and analyzed by HPLC (Agilent® HP 1100 series) equipped with a Zorbax® XDB C-18 reversed-phase chromatographic column (75 × 4.6 mm; 5 μm particle size). The mobile phase consisted of water and acetonitrile in a ratio of 42:58 (v/v). The column temperature was 45 °C, the flow rate was 1.2 mL min^{-1} and the detection wavelength was set at 220 nm. A correction factor was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium.

References and notes

1. Loftsson, T.; Duchêne, D. *Int. J. Pharm.* **2007**, *329*, 1.
2. Mura, P.; Faucci, M. T.; Bettinetti, G. P. *Eur. J. Pharm. Sci.* **2001**, *13*, 187.
3. Loftsson, T.; Frikdriksdottir, H. *Int. J. Pharm.* **1998**, *163*, 115.
4. Ribeiro, L.; Loftsson, T.; Ferreira, D.; Veiga, F. *Chem. Pharm. Bull.* **2003**, *51*, 914.
5. Gormley, G. *Biomed. Pharmacother.* **1995**, *49*, 319.
6. Andriole, G.; Bruchovsky, N.; Chung, L. W. K.; Matsumoto, A. M.; Rittmaster, R.; Roehrborn, C.; Russell, D.; Tindall, D. *J. Urol.* **2004**, *172*, 1399.
7. *The United States Pharmacopoeia/The National Formulary*; 30th ed.; US Pharmacopeial Convention: Rockville, 2007; pp 2133–2135.
8. *European Pharmacopoeia*; 4th ed.; Council of Europe: Paris, 2002; pp 1188–1189.
9. Korolkovas, A.; Cunha, B. C. A.; Carneiro, F. F. A.; Ferreira, E. I. *Dicionário Terapêutico Guanabara*; Guanabara Koogan: Rio de Janeiro, 1995.
10. Loftsson, T.; Hreinsdóttir, D.; Másson, M. *J. Inclusion Phenom. Macrocycl. Chem.* **2007**, *57*, 545.
11. Loftsson, T.; Hreinsdóttir, D.; Másson, M. *Int. J. Pharm.* **2005**, *302*, 18.
12. Omari, M. M. A.; Zughul, M. B.; Davies, J. E. D.; Badwan, A. A. *J. Pharm. Biomed. Anal.* **2006**, *41*, 857.
13. Loftsson, T.; Frikdriksdottir, H.; Sigurkdardottir, A. M.; Ueda, H. *Int. J. Pharm.* **1994**, *110*, 169.
14. Brewster, M. E.; Loftsson, T. *Adv. Drug Delivery Rev.* **2007**, *59*, 645.
15. Rama, A. C. R.; Veiga, F.; Figueiredo, I. V.; Sousa, A.; Caramona, M. *Braz. J. Pharm. Sci.* **2005**, *41*, 281.
16. Zingone, G.; Rubessa, F. *Int. J. Pharm.* **2004**, *291*, 3.
17. Veiga, F.; Teixeira-Dias, J. J. C.; Kedzierewicz, F.; Souza, A.; Maincent, P. *Int. J. Pharm.* **1995**, *129*, 63.
18. Higuchi, T.; Connors, K. A.. In *Advances in Analytical Chemistry Instrumentation*; Wiley Interscience: New York, 1965; Vol. IV.