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Research paper

Lyophilization monophase solution technique for improvement of the physicochemical properties of an anticancer drug, flutamide

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ABSTRACT

Flutamide (FLT), an anticancer drug for prostatic carcinoma, has poor aqueous solubility and low oral bioavailability. This study describes the ability of β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) to form complexes with flutamide with enhanced solubility and dissolution rate in vitro. FLT–CD lyophilized dispersions (LDs) were prepared via lyophilization monophase solution technique using tertiary butyl alcohol (TBA) as a cosolvent. FLT showed an A_L-type phase solubility diagram consistent with a linear increase in drug solubility as a function of CD concentration. Gas chromatography indicated that the LDs contain 0.02–0.03% w/w residual TBA. Based on the data from differential scanning calorimetry (DSC) and X-ray diffractometry (XRD), FLT was fully amorphous in 1:5 FLT-HPβCD LD as indicated by complete disappearance of FLT endothermic and diffraction peaks. The Fourier transform infrared (FTIR) spectra indicated that a FLT–CD interaction took place in the lyophilized complex. The particle sizes of 1:1 FLT-βCD and FLT-HPβCD LDs were 0.92 and 0.82 μm, with a high surface area (484.55 and 705.68 m^2/g) and porosity (769.46 and 1020.99 $e^{-3}ml/g$), respectively. The dissolution rate of FLT from its CD complexes was enhanced significantly. After 30 min in 0.1 N HCl, about 73% and 86% of FLT were dissolved from 1:5 FLT-βCD and FLT-HPβCD LDs, respectively, compared to only 13.45% of pure drug. No endothermic peak corresponding to FLT melting was detected in 1:5 FLT-HPβCD LD after storage at 20 °C and 45% relative humidity for 90 days thus indicating the stability of this binary system. These data suggest that cyclodextrins might be useful adjuncts in preparation of immediate-release formulations of FLT.

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1. Introduction

Prostate cancer has become one of the most common malignancies in the male population worldwide. Antiandrogenic agents are therapeutically effective for benign prostatic hypertrophy (BPH) and androgen dependent prostate cancer. Of the nonsteroidal antiandrogens, flutamide (FLT) is the only one presently recommended for monotherapy [1]. Flutamide is used increasingly as part of total androgen ablation therapy and in neoadjuvant treatment before radical prostatectomy [2].

The low bioavailability of FLT after oral formulations may be due to poor wettability, low aqueous solubility, poor permeability, rapid first pass hepatic metabolism and low concentration at the absorption surface [3]. Therefore, developing novel formulations that mitigate solubility and dissolution will produce higher concentrations of FLT in solution at the absorption site and hence may overcome the solubility-mediated poor bioavailability.

Various methods have been described to enhance the solubility of poorly soluble drugs (e.g. the use of pro-drugs, the addition of surfactants, salt selection, solid dispersions and particle size reduction) [4]. In addition, cyclodextrins, macrocyclic oligosaccharides, represent an important group of excipients used for this purpose. Cyclodextrins have a unique structure with a hydrophobic cavity and a hydrophilic exterior that can act as a host and form inclusion complexes with a variety of guest molecules. Upon complexation with an active pharmaceutical ingredient, cyclodextrins will increase the solubility of its sparingly soluble guest molecule. Cyclodextrin component often affords additional beneficial properties (e.g. stabilization of unstable active pharmaceutical ingredients and taste masking) [5,6].

Few studies have been performed to improve the solubility of FLT and enhance its dissolution rate by preparing its high energy solid dispersions. Coprecipitates prepared using different ratios of FLT with α-cyclodextrin and β-cyclodextrin via solvent technique revealed that β-cyclodextrin was more efficient than α-cyclodextrin in enhancing the drug dissolution rate [7]. In another study, FLT-HPβCD inclusion complex was prepared using solvent/freeze-drying technique in a completely aqueous solution. Results showed that the complex substantially increased the aqueous FLT solubility and improved its oral bioavailability in rats [3,8].

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The abovementioned solvent technique requires dissolution of both drug and cyclodextrin in one solution (water) followed by drying, for example, vacuum drying, spray drying or freeze-drying [9,10]. Freeze-drying is an industrially applicable method especially for heat labile drugs and biopharmaceutical compounds. However, low drug concentrations, tremendous amounts of water and excessive cyclodextrin would be required because of the low solubility of the hydrophobic drug in aqueous solution. This will make the preparation process time consuming [11] and energy wasting, excessive CD is also unbeneficial for humans [12].

Therefore, lyophilization monophase solution technique was developed as a suitable alternative procedure that could overcome the abovementioned demerits of the conventional freeze-drying. In this technique, TBA, which is miscible with water in any proportion, was used as an organic cosolvent to solubilize the hydrophobic drug while the hydrophilic carrier was dissolved in water then the mixed isotropic solution was lyophilized. TBA possesses a high vapor pressure (41.25 mm Hg at 25 °C), a high melting point (24 °C) and has a low toxicity [13]. Moreover, adding TBA to water results in formation of larger needle-shaped ice crystals with a higher surface area and porosity than round ice crystals that can facilitate sublimation. All these factors contribute TBA as an ideal freeze-drying medium that could be removed rapidly and completely by freeze-drying [14].

Lyophilization monophase solution technique was used to enhance the dissolution rate of the poorly soluble drugs; budesonide, salmeterol, ketoprofen and nitrendipine by complexation with βCD and HPβCD [15,16]. In both studies, the enhanced drug dissolution was only attributed to drug amorphization through inclusion complex formation.

However, the mechanism of increased dissolution rate of drugs by solid dispersion technique can be attributed to a number of factors such as drug amorphization, increased drug solubility, reduced particle size, higher degree of surface area and porosity [17].

Therefore, the objective of the present study is to prepare FLT–CD complexes at different molar ratios by the lyophilization monophase solution technique and to investigate the influence of these complexes on flutamide solubility and dissolution performance. The contribution of various FLT physicochemical parameters (e.g. solubility, crystallinity, particle size, surface area and porosity) in enhancing the drug dissolution was also investigated.

2. Materials and methods

2.1. Materials

Flutamide (FLT) was kindly donated by Archimica (Origgio, Italy). β-Cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD) and tertiary butyl alcohol (TBA) were purchased from Sigma–Aldrich (St. Louis, USA). All other chemicals were of analytical grade and used without further purification.

2.2. HPLC assay for flutamide

A reverse phase HPLC method was used for quantifying flutamide [18]. HPLC analysis was carried out with a Perkin Elmer series 200 chromatograph (Perkin Elmer, USA) using a Spheri-5, RP-18, 220 mm × 4.6 mm, 5 μm, column (Perkin Elmer, USA) and a UV detector. An isocratic solvent system consisting of 75:25 (v/v) methanol/water was used at a flow rate of 1 ml/min, an injection volume of 20 μl and the peaks were detected at 304 nm. Under these experimental conditions, the total run time was approximately 6 min and the retention time was 3.5 min. Calibration curves (peak area vs. drug concentration) were linear ($R^2 > 0.999$) over the FLT concentration range of 0.6–60 μg/ml.

2.3. Phase solubility study

Aqueous solubility of FLT in presence of either βCD or HPβCD was carried out according to the method described by Higuchi and Connors [19]. An excess amount of FLT was added to 10 ml of aqueous solutions containing increasing concentrations of βCD or HPβCD (0, 3, 6, 9, 12, 15 and 18 × 10^{-3} M) in screw-capped vials. The suspensions were shaken in a thermostatically controlled water bath (GFL, type 1083, Gmbh & Co., Burgwedel, W. Germany) at 37 ± 0.5 °C for 24 h. After equilibrium has been attained (2 days), aliquots were withdrawn, filtered through 0.45-μm membrane filter, suitably diluted and analyzed for FLT using HPLC at 304 nm. Each experiment was carried out in triplicate. Assuming the formation of a complex with a 1:1 stoichiometric ratio, the apparent stability constant (K_s) of 1:1 FLT–CD complexes was calculated from the linear phase solubility diagram obtained by plotting the molar concentration of FLT in the solution vs. CD molar concentration according to the equation:

$$K_s = \text{slope}/[S_0(1 - \text{slope})]$$

where S_0 is intrinsic FLT aqueous solubility in the absence of cyclodextrin.

The solubilization efficiency of cyclodextrins was calculated as the ratio of FLT aqueous solubility at the highest cyclodextrin concentration used (18 mM) and FLT intrinsic solubility in pure water.

2.4. Preparation of FLT–CD lyophilized dispersions and physical mixtures

The calculated amount of cyclodextrin was dissolved in 5 ml water and mixed with FLT/TBA solution (300 mg/5 ml) in 50-ml vials. Immediately after mixing, the vials were frozen at −80 °C for 4 h followed by placing them in a Cryodos-50 lyophilizer (Telstar Cryodos, Spain) with a condenser temperature of −70 °C. Lyophilization was performed at a pressure of 40 mbar and a shelf temperature of −40 °C for 1 day followed by a secondary drying at 25 °C for another day. After removing the samples from the freeze-drier, they were placed in a desiccator over P_2O_5 at 4 °C until testing. The corresponding physical mixtures (PMs) were prepared by homogenous blending of accurately weighed amounts of the drug and cyclodextrin in a mortar and stored at room temperature in hermetically sealed bottles until use.

2.5. Characterization of FLT lyophilized dispersions

2.5.1. Residual TBA determination

The amount of residual TBA in lyophilized dispersions was determined using AutoSystem XL gas chromatograph (GC) with RX5 column crossbond 5% diphenyl-95% dimethyl polysiloxane 30 m, 0.32 m ID with a film thickness of 0.25 μm (Perkin Elmer, USA). A known weight of the lyophilized sample was immediately dispersed in 2 ml distilled water and GC quantificated the concentration of TBA in 1 μl of the vapor. The injection temperature was 200 °C. Oven was heated from 70° to 100 °C at a rate of 2 °C/min and a flame ionization detector operating at 150 °C yielded quantitative data. Control experiments showed that the peak areas were not affected by presence of the drug or carriers. Calibration curves of pure TBA/water mixtures were used for all experiments.

2.5.2. Differential scanning calorimetry (DSC)

DSC thermograms of pure materials, PMs and LDs were recorded by DSC 6 differential scanning calorimeter (Perkin Elmer, USA). Samples (2–4 mg) were placed in sealed aluminum pans and heated at 10 °C/min under a nitrogen atmosphere (flow rate 20 ml/min) in the 30–400 °C range. An empty aluminum pan was

used as a reference. The equipment was periodically calibrated with indium.

The heat of fusion of crystallized drug in a LD was calculated from the peak area of the melting endotherm. The heat of fusion of pure crystalline drug was determined in a separate experiment. The ratio of these fusion energies was used to calculate the percent crystallinity of drug in the LDs and PMs using the following equation:

$$\%\ \text{Crystallinity} = 100 \times \frac{\Delta Hs}{\Delta Hc \times C}$$

where ΔHs and ΔHc are enthalpies of fusion of the sample and pure drug, respectively, and C is the weight fraction of drug in the mixture assuming that the pure drug was 100% crystalline [20].

2.5.3. Physical stability

To determine the physical stability of LDs, samples were placed in a climate chamber of 20 °C and 45% relative humidity (RH). After 90 days, the % crystallinity of FLT in the samples was determined by means of differential scanning calorimetry (DSC).

2.5.4. Powder X-ray diffractometry (XRD)

The X-ray diffractograms of pure materials and LDs were carried out using XRD-7000 X-ray diffractometer (Shimadzu, Japan) where Cu $K\alpha_1$ radiation was selected by a Ni monochromator. The scanning rate employed was 2°/min over a diffraction angle of 2θ and range of 5–60°, operated at a voltage of 30 kV and a current of 30 mA, the scan step size was 0.018 (2θ). The analysis was carried out at room temperature under ambient conditions.

2.5.5. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of pure materials, PMs and LDs were recorded using a Spectrum RXI FT-IR spectrophotometer (Perkin Elmer, USA) according to the KBr disk technique, and IR measurements were performed in transmission in the scanning range of 4000–500 cm^{-1} at ambient temperature.

2.5.6. Scanning electron microscopy (SEM)

The surface morphology of drug and LDs was examined by means of a JEM-100S scanning electron microscope (Joel, Japan). Double-sided adhesive tape was placed on an aluminum specimen holder upon which a small amount of powdered samples was deposited. The particles were coated with approximately 10–20 nm gold for 20 s using a sputter coater. Scans were performed at an acceleration voltage of 10 kV.

2.5.7. Particle size analysis

The particle size of pure drug and LDs was determined using model 1064 liquid laser diffraction particle size analyzer (Cilas, France). A suitable amount of sample was transferred to the dispersion medium of 0.1 N HCl. The medium was agitated at 100 rpm. Particle size was expressed as the equivalent number diameter.

2.5.8. Surface area and porosity analysis

Specific surface area and porosity of pure drug and LDs were measured using the NOVA 1000 series surface area analyzer (Quantachrome, USA). A known weight of powder was added to a 12 mm Quantachrome bulb sample cell and degassed for 3 h prior to analysis. A 5-point nitrogen adsorption isotherm at 77 K was measured, and the sample was then analyzed by the NOVA enhanced data reduction software via the Brunauer, Emmett and Teller (BET) theory of surface area [21].

2.5.9. In vitro dissolution study

FLT dissolution behavior was evaluated using the USP XXIV dissolution rate apparatus II (Pharmatest, Germany) at a stirring rate of 100 ± 2 rpm. Powder samples containing 60 mg of pure FLT or its equivalent amount of lyophilized dispersions or physical mixtures were placed in 900 ml of dissolution fluid (0.1 N HCl, pH 1.2) at 37 ± 0.5 °C for 2 h. At predetermined time intervals, 5 ml of samples were withdrawn and immediately replaced with an equal volume of prewarmed dissolution medium. All samples were run in triplicate, filtered through 0.45 μm membrane filter, and the amount of dissolved FLT was analyzed by HPLC at 304 nm. The percentage cumulative amount of drug dissolved was plotted against time.

3. Results and discussion

3.1. Phase solubility study

The solubility method is useful for studying inclusion compound of poorly soluble drugs with CDs in water because it gives not only the solubilizing ability of CDs but also the stability constant (K_s) of the complexes by analyzing the solubility curves [19]. The phase solubility profiles for the FLT–CD systems were presented in Fig. 1. The diagram showed that the aqueous solubility of the drug increased linearly as a function of CD concentration, over the entire concentration range studied. The solubility curve was regarded as a straight line (A_L type) [19]. Because of this linear host–guest correlation with slope of less than 1 (i.e. 0.0396 and 0.016 for FLT-HPβCD and FLT-βCD, respectively), it was suggested that the complexes formed were of the first order with regard to the host molecule concentrations [22]. The stoichiometry 1:1 apparent stability constant (K_s) of the complex was calculated from the slope of the straight line of the phase solubility diagram to be 205.82 and 521.93 M^{-1} for βCD and HPβCD, respectively. The solubilization efficiency (SE) values revealed that the solubilizing power of HPβCD (9.87) toward the drug was higher than that of βCD (4.56) with more than 9.87-fold increase of drug intrinsic solubility in presence of 18 mM carrier compared to 4.56-fold increase in case of βCD [23].

3.2. Optimization of TBA/water ratio

Using TBA/water as a cosolvent for the preparation of the hydrophobic FLT with the hydrophilic carrier CD, a high drug concentration could be achieved due to its excellent solubility in TBA. This helped to accelerate complex formation and shortened the lyophilization cycle [24]. To produce isotropic solution of the drug with the hydrophilic carrier, the optimal TBA/water ratio 1/1 (v/v) was found to guarantee the physical stability of drug and carrier. This ratio is in accordance with that used to prepare cyclodextrin

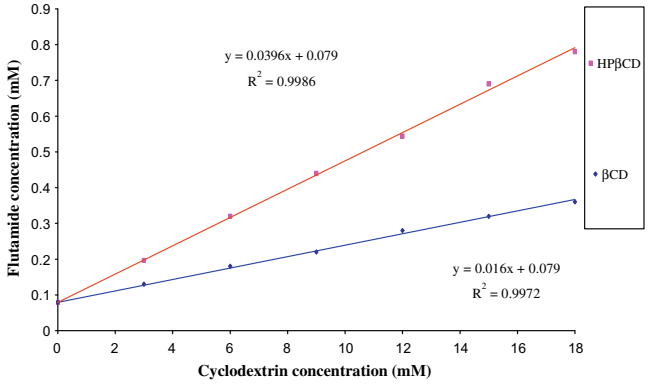

Fig. 1. Phase solubility diagram of flutamide in aqueous solutions of cyclodextrins at 37 °C.

inclusion complexes with salmeterol and budesonide by the lyophilization monophase solution method [15].

3.3. Characterization of FLT lyophilized dispersions

3.3.1. Residual solvent determination

Based upon its high volatility, high viscosity, crystal morphology and low reactivity, TBA is determined to be a suitable freeze-drying medium for poorly water soluble drugs. The controlling of residual TBA was needed though TBA is a low toxic organic solvent and has little detriment to human body. According to the International Conference on Harmonization (ICH) guidelines for residual solvents, solvents are divided into three different categories: classes 1–3 solvents, with class 1 indicating extremely high toxicity and class 3 indicating a very low toxicity. Although not listed in the ICH guidelines, TBA is likely to fall in the category of a class 3 solvent based on its similarity of LD_{50} toxicity data for other class 3 solvents with a maximum daily dose of 50 mg. Therefore, the low level of TBA in the lyophilized cakes should not be harmful to both animal and human [13,24].

Gas chromatography confirmed that there are 0.02% and 0.03% w/w residual of TBA in the 1:1 FLT-βCD and FLT-HPβCD LDs, respectively, which was much lower than the toxic level. The low level of TBA in the lyophilized dispersions results from its ability to form high surface area crystals and from the fact that the intermolecular forces among TBA molecules are not as strong as those of water. This allows TBA to sublime more completely and easily than water [25,26].

3.3.2. Differential scanning calorimetry (DSC)

DSC is the most highly regarded thermoanalytical method used frequently to detect the amount of crystalline material. The thermograms of pure components and different FLT–CD binary systems are shown in Figs. 2a and 2b. The DSC thermogram of FLT was typical of a crystalline substance with a sharp endothermic peak at 113.21 °C corresponding to its melting point that was in accordance with a previous report [7].

Liberation of crystal water from the cavity of βCD (14.5% as mass fraction) or HPβCD (11.9% as mass fraction) was observed as broad endothermic peaks around 85° and 70 °C, respectively [15,16]. The DSC thermograms of 1:1 FLT–CD systems showed two peaks; one sharp peak corresponding to FLT melting and the second broad peak corresponding to CD dehydration. The enthalpy of drug fusion decreased sharply with the incorporation of the carrier indicating that the drug lost an appreciable percentage of its crystallinity (Table 1). Upon increasing the carrier ratio in the FLT–CD 1:3 LDs, the characteristic peak of FLT appeared nearly at its melting point with a significant reduction in enthalpy of fusion and percentage of crystallinity. This modification of the DSC profile of the drug can be assumed as a proof of interactions between the components of the respective binary system. Total disappearance of the drug thermal profile was observed in the 1:5 FLT-HPβCD LD with a complete loss of its crystallinity thus indicative of drug amorphization and through true inclusion complex formation [15]. While the drug peak was still detectable in the 1:5 FLT-βCD LD but with a significant reduction of drug crystallinity assuming a partial dispersion at a molecular level in the solid product, but did not seem to be indicative of a true inclusion complex formation [27].

The thermograms of the physical mixtures of FLT–CD systems showed both FLT and CD endotherms. However, enthalpy values

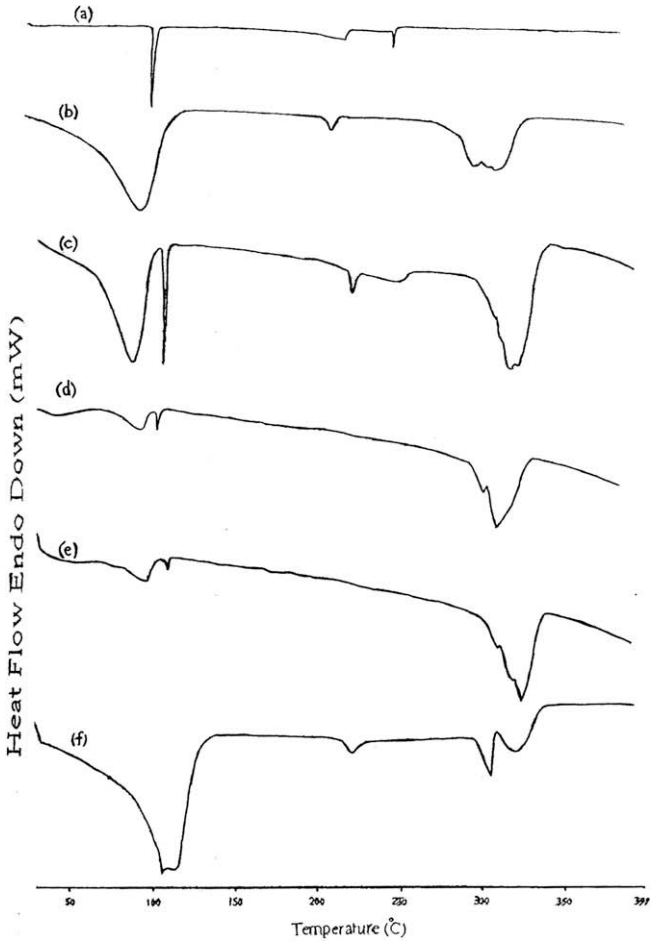

Fig. 2a. DSC thermograms of FLT-βCD systems: pure FLT (a), βCD (b), FLT-βCD 1:1 LD (c), FLT-βCD 1:3 LD (d), FLT-βCD 1:5 LD (e), FLT-βCD 1:5 PM (f).

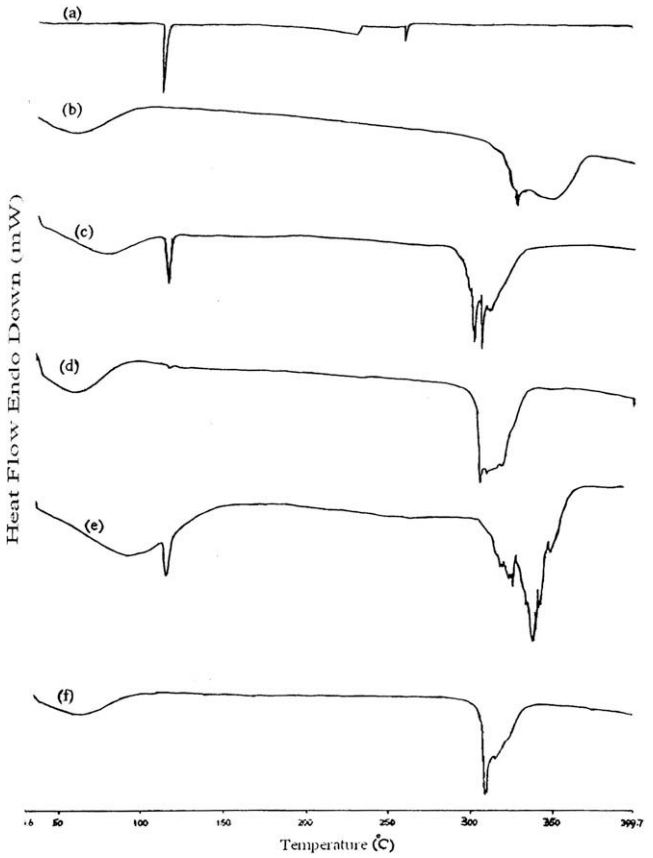

Fig. 2b. DSC thermograms of FLT-HPβCD systems: pure FLT (a), HPβCD (b), FLT-HPβCD 1:1 LD (c), FLT-HPβCD 1:3 LD (d), FLT-HPβCD 1:3 PM (e), FLT-HPβCD 1:5 LD (f).

Table 1
Thermotropic parameters of FLT and its lyophilized dispersions before and after storage for 3 months at 20 °C and 45% relative humidity.

Formula	Peak (°C)		ΔH (J/g)		% Crystallinity	
	AP[a]	AS[b]	AP[a]	AS[b]	AP[a]	AS[b]
FLT	113.21	113.11	83.86	83.86	100	100
FLT-βCD 1:1	112.43	112.31	10.03	15.3	59.78	92.62
FLT-βCD 1:3	112.04	112.47	3.42	5.99	51.04	89.39
FLT-βCD 1:5	112.08	112.56	1.98	2.78	47.12	66.44
FLT-HPβCD 1:1	112.40	112.23	8.32	10.60	62.00	79.00
FLT-HPβCD 1:3	110.67	112.31	0.39	0.47	7.83	9.24
FLT-HPβCD 1:5	–	–	–	–	–	–

[a] AP: immediately after preparation.
[b] AS: after storage for 3 months at 20 °C and 45% relative humidity.

associated with FLT melting were much lower than that of the crystalline drug. This indicated a partial loss of crystallinity and occurence of an interaction with CD as a typical consequence of the modification of the pre-existing hydrogen bonds of the crystalline drug [28]. Moreover, the inclusion could be formed during the course of heating or the drug corresponded to only a small portion of the mixture total mass and the CD might partially mask the detection of the endothermic peak of drug [15].

3.3.3. Physical stability

The dissolution behavior of solid dispersions must remain unchanged during storage. The best way to guarantee this is by maintaining their physical state and molecular structure. For optimal stability of amorphous solid dispersions, the molecular mobility should be as low as possible. However, solid dispersions, partially or fully amorphous, are thermodynamically unstable and will have a natural tendency to crystallize, because the crystalline state has a lower energy compared to amorphous material. However, amorphous material can be kinetically stable, which implies that the equilibrium state, i.e. crystalline, is not reached within the timeframe of the experiment or shelf life of the product. Therefore, the physical state should be monitored because changes therein are likely to alter the drug release [29].

The results of the stability study of FLT–CD LDs stored at 20 °C and 45% relative humidity for 90 days were shown in Table 1. The influence of FLT–CD molar ratio on the physical stability was investigated. The drug fusion enthalpy of 1:1 FLT-HPβCD LD was increased from 8.32 to 10.60 J/g after storage with a subsequent increase of FLT crystallinity from 62% to 79%. Reducing the drug proportion in 1:3 FLT-HPβCD LD increased the FLT crystallinity from 7.83% of the freshly prepared LD to only 9.25% after storage. From the abovementioned results, decreasing the drug load was found to reduce the tendency for recrystallization. Similar results were observed with FLT-βCD LDs. Firstly, the diffusion distance for separate drug molecules to form amorphous or crystalline particles is larger for lower drug contents. Hence, the formation of a separate drug phase is significantly retarded. Secondly, low drug contents minimize the risk of exceeding the solid solubility. When the solid solubility is lower than the drug load, there is a driving force for phase separation. And finally, if drug–matrix interaction increases stability, then also low drug contents are preferred, since in that case, drug–drug contacts will be rare and drug–matrix contacts omnipresent [30].

No peak corresponding to FLT melting was detected in the 1:5 FLT-HPβCD LD after storage. Thus, 100% drug amorphization was maintained. The high stability of this binary system could be explained by drug-carrier hydrogen bonding existed in this LD leading to the formation of a more stable inclusion complex that inhibited drug recrystallization. Moreover, in solid dispersions containing crystalline particles, these particles form nuclei that can be the starting point for further crystallization [29].

3.3.4. Powder X-ray diffractometry (XRD)

XRD analysis was performed to confirm the results of the DSC study. XRD patterns of FLT and FLT-βCD or FLT-HPβCD LDs (Figs. 3a and 3b) revealed that FLT is a crystalline compound showing a very strong sharp diffraction peak at 2θ of 8.726° while other peaks are present at a lower intensity. The XRD patterns of βCD revealed several diffraction peaks that are indicative of their crystalline character [31], while a hollow pattern was recorded for HPβCD that proved its amorphous state. The lyophilized dispersions presented diffraction patterns quite similar to the characteristic FLT peaks but broad with much lower intensity thus confirming that in these systems, there is no formation of a true inclusion complex. Such a decrease in drug crystallinity can be explained by the presence of reciprocal interactions in the solid state between host and guest [32].

On the other hand, a total drug amorphization was observed in the XRD profile of FLT-HPβCD 1:5 LD, where only two broad peaks corresponding to the diffraction pattern of HPβCD were recorded while peaks of FLT crystals were completely disappeared thus suggesting that HPβCD inhibited the crystallization of FLT through the formation of inclusion complexes, and that HPβCD was more efficient than βCD for FLT amorphization.

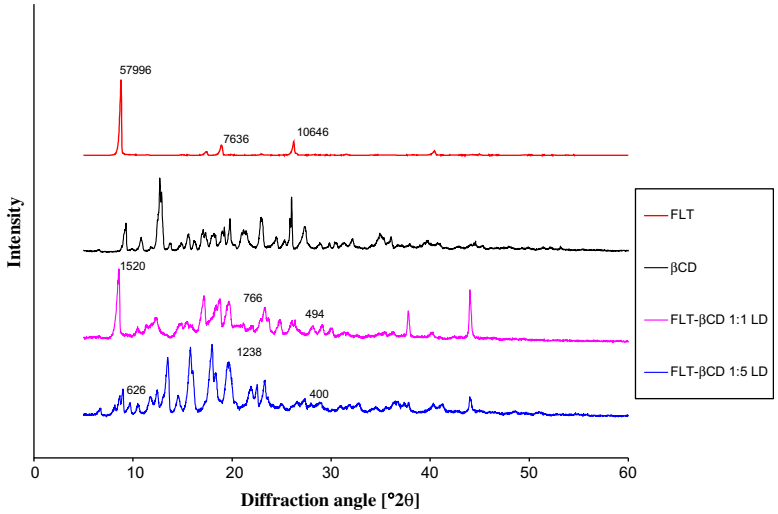

Fig. 3a. X-ray diffractograms of flutamide-βCD lyophilized dispersions.

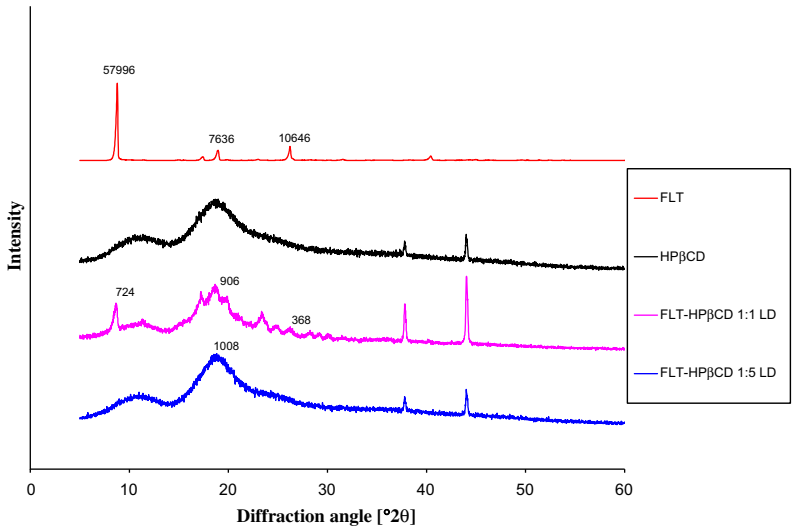

Fig. 3b. X-ray diffractograms of flutamide-HPβCD lyophilized dispersions.

These results confirmed that the partial or complete loss of drug crystallinity was not merely a thermal artifact caused during the DSC heating cycle and so conversion to an amorphous form was strongly suggested through the inclusion complex formation.

3.3.5. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of FLT complexes with βCD or HPβCD compared with that of their PMs and pure drug are shown in Figs. 4a and 4b, respectively. The characteristic peak of FLT at 3360 cm^{-1} corresponding to its amino group was detected in 1:1 FLT–CD LDs. Increasing the carrier content to 1:3 and 1:5 FLT-HPβCD resulted in a shift in the drug peak to lower values of 3341 and 3295 cm^{-1}, respectively. While in case of βCD, the drug peak was shifted to 3359 and 3261 cm^{-1}. Furthermore, the carbonyl stretching peak at 1718 cm^{-1} of FLT was also recorded in 1:1 FLT-HPβCD LD. Decreasing the drug content in 1:3 and 1:5 FLT-HPβCD, this

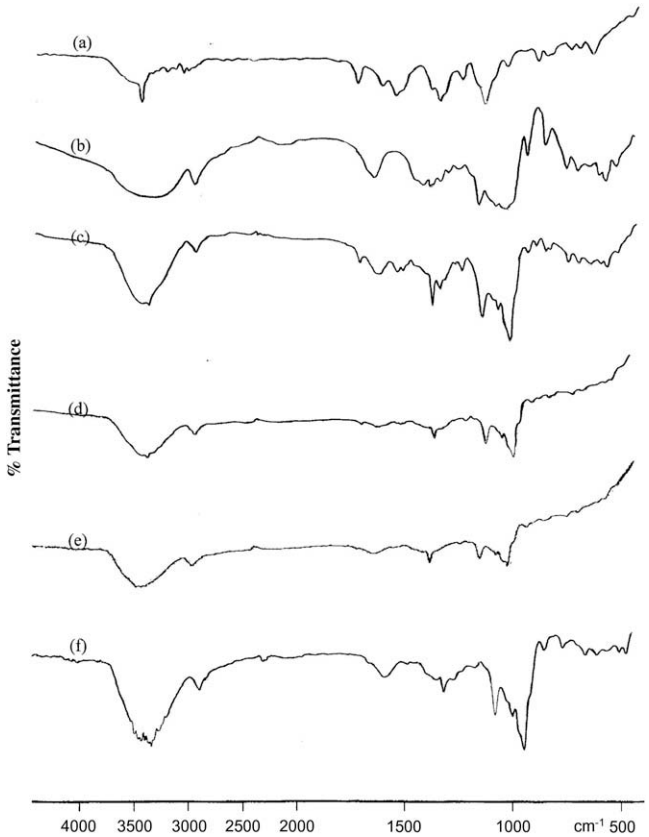

Fig. 4a. FT-IR transmission spectra of FLT-βCD systems: pure FLT (a), βCD (b), FLT-βCD 1:1 LD (c), FLT-βCD 1:3 LD (d), FLT-βCD 1:5 LD (e), FLT-βCD 1:5 PM (f).

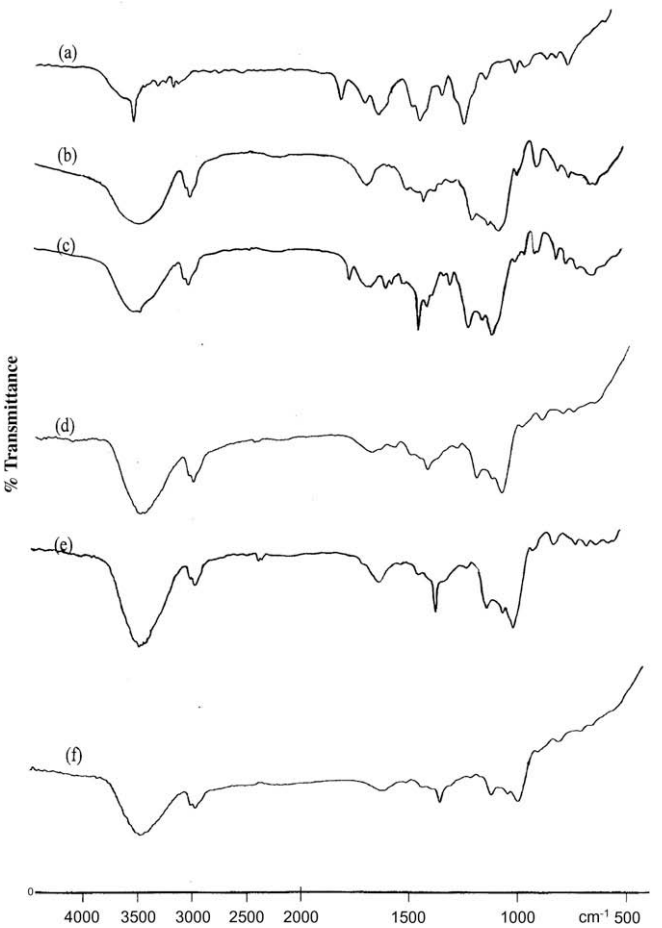

Fig. 4b. FT-IR transmission spectra of FLT-HPβCD systems: pure FLT (a), HPβCD (b), FLT-HPβCD 1:1 LD (c), FLT-HPβCD 1:3 LD (d), FLT-HPβCD 1:3 PM (e), FLT-HPβCD 1:5 LD (f).

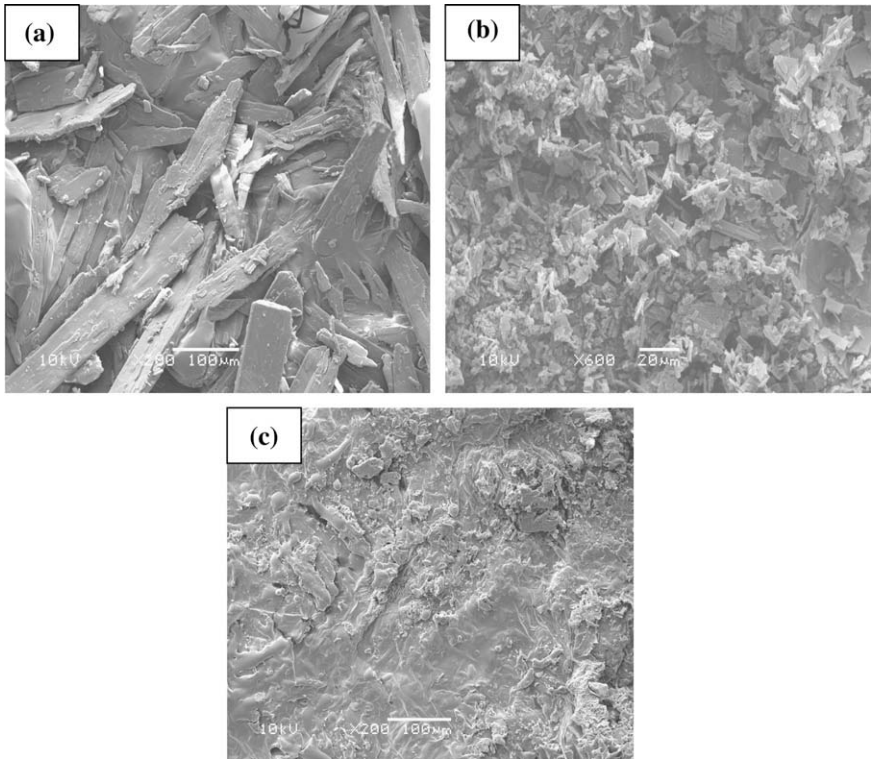

Fig. 5. Scanning electron micrographs of pure FLT (a), FLT-βCD 1:1 LD (b), FLT-HPβCD 1:1 LD (c).

peak was shifted to lower values of 1646.8 and 1651 cm^{-1}, respectively. These results indicated a change in the environment of carbonyl group of the drug as a consequence of the interaction with HPβCD. On the other hand, the same peak was present in position in all ratios of FLT-βCD LDs [15].

The band shape attributed to the stretching vibration of the carbonyl group was appeared broader and shifted to lower values, suggesting a weak interaction (intermolecular hydrogen bonds) between the drug and carrier after formation of 1:1 inclusion complex [33]. The spectra of PMs were that resulting from simple superposition of each component.

3.3.6. Scanning electron microscopy (SEM)

The SEM images for pure FLT, 1:1 FLT-βCD and FLT-HPβCD LDs are shown in Figs. 5a–c, respectively. Pure drug image showed plate-like crystals whereas on using βCD, the characteristic FLT crystals were clearly detectable, but crystal sizes were smaller thus confirming the presence of crystalline drug. This fact was observed in lyophilized dispersions between βCD and other poorly water soluble drugs [34]. However, the micrograph of FLT-HPβCD LD did not show single entity of drug crystals but showed homogeneity, hinting FLT molecules could be dispersed uniformly in the carrier matrix of LDs assuming amorphous solid dispersion state or the drug was incorporated into an inclusion complex. These findings were in agreement with the drug crystallinity values determined by DSC study (Table 1).

3.3.7. Particle size analysis

It is known that in glass, solid solutions and amorphous dispersions, the particle size is reduced to a minimum level. After carrier dissolution, the drug is molecularly dispersed in the dissolution medium resulting in an enhanced dissolution rate [35].

Literature lacks any data about the effect of cyclodextrins on the particle size of drug in solid dispersions. Therefore, our study gave a special emphasis to particle size of FLT in its lyophilized dispersions. The average particle size of FLT crystals was 2.96 μm. The lyophilization technique was found to reduce the particle size of the drug in its CD LDs. In addition, the decrease in the particle size might be a factor of the carrier nature. The particle size of the 1:1 FLT-βCD or FLT-HPβCD LDs was 0.92 or 0.82 μm, respectively (Table 2). Increasing the FLT/CD ratio to 1:3 increases the particle size of the same LDs to 0.96 or 0.98 μm, respectively. The extent of particle size increase in case of HPβCD is higher than that of βCD.

3.3.8. Surface area and porosity analysis

An important factor that influences the dissolution rate is the available surface area of the active pharmaceutical ingredient (API).

Table 2
Particle size, specific surface area and porosity measurements for FLT lyophilized dispersions (values are mean ± SD, $n = 3$).

Formula	Particle size (μm)	Specific surface area (m^2/g)	Total pore volume (e^{-3} ml/g)	Average pore radius (Å)
FLT	2.96 ± (0.03)	233.42 ± (10.06)	339.64 ± (5.71)	29.10 ± (0.63)
FLT-βCD 1:1	0.92 ± (0.03)	484.55 ± (12.12)	769.46 ± (14.9)	28.77 ± (0.71)
FLT-βCD 1:3	0.96 ± (0.02)	420.34 ± (21.6)	729.45 ± (9.74)	28.92 ± (0.35)
FLT-βCD 1:5	1.13 ± (0.03)	393.89 ± (15.83)	655.67 ± (17.76)	29.21 ± (0.38)
FLT-HPβCD 1:1	0.82 ± (0.04)	705.68 ± (8.64)	1020.99 ± (16.15)	28.94 ± (0.48)
FLT-HPβCD 1:3	0.98 ± (0.08)	643.39 ± (17.73)	949.53 ± (24.41)	29.65 ± (0.16)
FLT-HPβCD 1:5	1.14 ± (0.06)	618.45 ± (14.1)	905.78 ± (12.73)	28.89 ± (0.5)

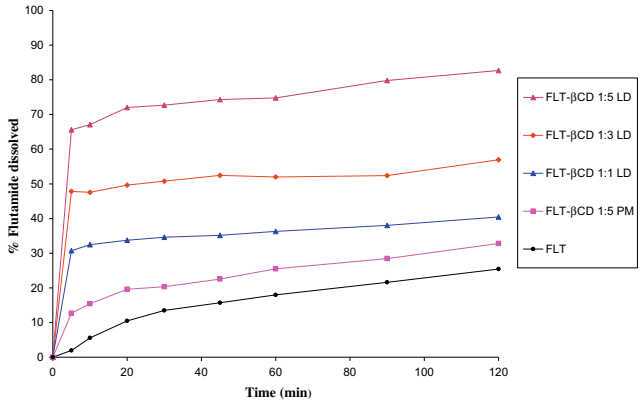

Fig. 6a. Dissolution profile of flutamide from different FLT-βCD systems in 0.1 N HCl at 37 °C.

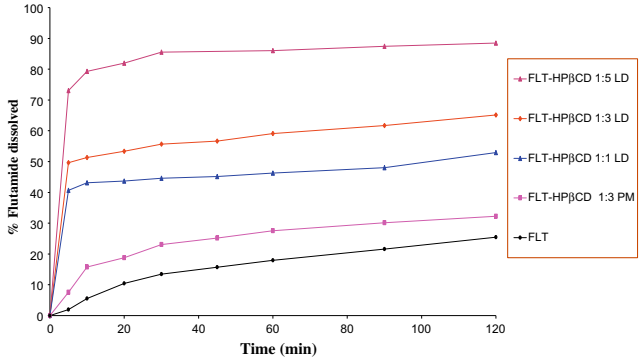

Fig. 6b. Dissolution profile of flutamide from different FLT-HPβCD systems in 0.1 N HCl at 37 °C.

The specific surface area of FLT crystals was 233.42 m^2/g with a total pore volume of 339.64 e^{-3} ml/g. The specific surface area was increased approximately by three folds (705.68 m^2/g) with a total pore volume of 1020.993 e^{-3} ml/g when the drug formed an inclusion complex with HPβCD in the ratio of 1:1 (Table 2). The FLT-βCD 1:1 LD exhibited a higher specific surface area and pore volume than those of FLT by more than two folds. Such a high surface area and porosity indicated that the lyophilized powder particles were highly porous due to the channels created as the solvents were removed during the sublimation phase of lyophilization process [36]. Both parameters were found to quietly decrease upon increasing the FLT/CD molar ratio in 1:3 and 1:5 FLT–CD LDs due to the particle size increase.

3.3.9. In vitro dissolution study

The dissolution profiles for the systems under study are presented in Figs. 6a and 6b. For their evaluation, four parameters, dissolution efficiency calculated after 60 min (%DE_{60}), percentage of dissolved drug after 30 min (PD_{30}), relative dissolution rate after 5 min (RDR_5) and time required to dissolve 25% of drug ($T_{25\%}$), were measured for all products studied (Table 3). The dissolution rate of pure FLT was very slow that after 120 min about 19% of the drug was dissolved. This poor dissolution behavior of the drug could be explained on the basis of its poor wettability and/or agglomeration.

FLT dissolution was enhanced when physically mixed with CDs FLT with respect to FLT alone. This fact can be attributed to the improved wettability of PMs due to the presence of cyclodextrins that reduce the interfacial tension between the water insoluble drug particles and the dissolution medium as well as, in early stages of the dissolution process, since CDs dissolve in a short time, CD molecules have local solubilization action operating in the microenvironment or the hydrodynamic layer surrounding the particles of FLT. This action resulted in an in situ inclusion process causing a rapid increase in the amount of dissolved drug [37].

It was obvious that the lyophilized dispersions showed marked increase in FLT dissolution, showing FLT relative dissolution rate (RDR_5) of 15.66 ± 0.25 and 20.77 ± 1.03 for 1:1 FLT-βCD and FLT-HPβCD LDs, respectively. Such increase could be attributed mainly to the formation of soluble inclusion complexes of the drug with CDs and the high energetic amorphous state or reduction of the crystallinity following complexation as reported previously [38]. Additionally, Betageri and Makarla [39] stated that the marked increase in the dissolution rate might be due to the formation of solid solution of the drug in the lyophilized products as a result of the complete inclusion of the drug into the CD cavities. The particle size was reduced to the molecular size when the carrier brought the drug into the dissolution medium, leading to fast dissolution. This is in complete accordance with our physicochemical characterization where the particle size of FLT was reduced in both CD LDs exhibiting high specific surface area and porosity compared to pure drug (Table 2).

The solid lyophilized dispersions prepared using HPβCD exhibited superior enhancement in FLT dissolution compared with that prepared using the parent βCD. The dissolution efficiencies after 60 min (DE_{60}) for 1:5 FLT-βCD and FLT-HPβCD LDs were 68.93% and 79.69%, respectively. This could be explained on the basis of greater water solubility, better wetting ability and higher complexing power of βCD derivatives toward the drug in the solid state. This reflects the inability of the parent βCD to promote true inclusion complexation with FLT as confirmed by DSC and XRD studies. This could result from the highly hydrophobic property of the drug and the low water solubility of βCD [40]. This was also in accordance with the higher stability constant of FLT-HPβCD complex re-

Table 3
Dissolution parameters of FLT, physical mixtures and lyophilized dispersions in 0.1 N HCl at 37 °C (values are mean ± SD, $n = 3$).

Formula	%DE_{60}[a]	RDR_5[b]	PD_{30}[c]	$T_{25\%}$[d]
FLT	10.83 ± (0.42)	1	13.45 ± (0.50)	120 ± (1.11)
FLT-βCD 1:1 LD	32.76 ± (1.00)	15.66 ± (0.25)	34.61 ± (0.82)	4 ± (0.3)
FLT-βCD 1:3 LD	47.94 ± (0.61)	24.39 ± (0.34)	50.75 ± (0.99)	3 ± (0.1)
FLT-βCD 1:5 LD	68.93 ± (0.58)	33.45 ± (0.37)	72.72 ± (0.80)	2 ± (0.02)
FLT-βCD 1:5 PM	16.42 ± (0.39)	6.48 ± (0.20)	17.34 ± (0.28)	79 ± (0.46)
FLT-HPβCD 1:1 LD	42.43 ± (0.39)	20.77 ± (1.03)	44.62 ± (0.58)	3 ± (0.06)
FLT-HPβCD 1:3 LD	52.61 ± (0.27)	25.34 ± (0.56)	55.66 ± (0.66)	2 ± (0.06)
FLT-HPβCD 1:3 PM	20.28 ± (0.31)	3.85 ± (0.20)	23.10 ± (0.58)	45 ± (0.83)
FLT-HPβCD 1:5 LD	79.69 ± (0.53)	37.27 ± (0.68)	85.54 ± (0.95)	1 ± (0.18)

[a] %DE_{60}: area under the dissolution curve up to 60 min.
[b] RDR_5: ratio of FLT dissolved from lyophilized dispersion to that of drug alone at 5 min.
[c] PD_{30}: percentage of FLT dissolved at 30 min.
[d] $T_{25\%}$: time required to dissolve 25% of FLT.

vealed in the phase solubility studies in addition to its lower particle size and higher surface area and porosity compared to FLT-βCD complex. The increased dissolution rate of FLT from its lyophilized formulations with βCD could be attributed to the formation of amorphous drug upon freeze-drying with cyclodextrin rather than due to formation of inclusion complexes [7].

The influence of FLT/CD molar ratio on the dissolution was clear, where the drug dissolution was enhanced on increasing the cyclodextrin proportion. This pronounced effect for the molar ratio in the lyophilized products was observed due to better dispersion and/or inclusion of the drug with increasing the cyclodextrin molar ratio during preparation [41]. According to the results of the particle size analysis, it was noticed that the increase in dissolution rate of FLT from its complexes with CD may not be merely due to the reduction in particle size. Although the addition of HPβCD reduced the particle size of pure drug from 2.96 μm to 0.82, 0.98 and 1.14 μm in the 1:1, 1:3 and 1:5 FLT-HPβCD LDs, respectively, the drug dissolution rate increased as the proportion of the carrier increased. Similar results were obtained with βCD.

4. Conclusions

Results from the present study suggest that the low oral bioavailability of FLT could be well circumvented by lyophilization monophase solution technology. Complexation with βCD and HPβCD significantly improved the dissolution rate of FLT through a number of factors such as drug amorphization, increased drug solubility, reduced particle size, increased surface area and higher degree of porosity. It was observed that FLT-HPβCD complex was more stable and soluble in water than FLT-βCD complex thus HPβCD offers a more useful tool rather than βCD in terms of improving the physicochemical properties of FLT.

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