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ORIGINAL ARTICLE

Lyophilized flutamide dispersions with polyols and amino acids: preparation and in vitro evaluation

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Abstract

Context: Flutamide (FLT) has poor aqueous solubility and low oral bioavailability. *Objective:* Lyophilization monophase solution was used for preparing lyophilized dispersions of FLT with polyols and amino acids to increase its poor dissolution. *Methods*: Physical properties and dissolution behavior of their physical mixtures and lyophilized dispersions were investigated. *Results and discussion*: The carriers increased the aqueous solubility of FLT but to a limited extent with arginine and glycine showing a linear A_L-phase solubility diagrams. Gas chromatography indicated that residual tertiary butyl alcohol was in range of 0.007–0.023% (w/w) in the dispersions. In all dispersions, the crystal structure of FLT was confirmed using differential scanning calorimetry, X-ray diffractometry, and scanning electron microscopy. However, the percent drug crystallinity was found to decrease with increasing the carrier content. Infrared spectroscopy revealed no interaction between drug and carriers. The particle size of FLT dispersions ranged between 0.61 and 1.81 μm, with a high surface area (293.93–465.37 m^2/g) and porosity (447.69–754.33 e^{-3} mL/g). In addition, the poor flow properties of FLT were improved but to a limited extent. FLT dissolution from the dispersions was enhanced with 46.35% and 36.43% of FLT dissolved after 30 minutes from 1:5 FLT–mannitol and FLT–trehalose dispersions, respectively, compared with only 13.45% of pure FLT. On the other hand, after 30 minutes 38.57% and 46.78% of FLT was dissolved from 1:3 FLT–arginine and FLT–glycine dispersions, respectively. *Conclusion*: These data suggest that polyols and amino acids might be useful adjuncts in preparation of immediate-release formulations of FLT.

Key words: Amino acids, lyophilization, monophase solution, particle size, polyols, surface area and porosity

Introduction

Antiandrogenic agents are therapeutically effective for benign prostatic hypertrophy and androgen-dependent prostate cancer. Flutamide (FLT) is the only one of the nonsteroidal antiandrogens presently recommended for monotherapy of prostatic carcinoma[1]. 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[2].

In a previous study, FLT lyophilized dispersions (LDs) with βCD and HPβCD were prepared using lyophilization monophase solution technique. Results showed that complexation with cyclodextrins significantly improved the dissolution rate of FLT[3].

Some sugars have been recommended and employed as carriers for solid dispersions. D-Mannitol, a water-soluble polyol, was selected as a vehicle in the preparation of solid dispersions of many poorly soluble drugs such as triamterene[4], ofloxacin[5], nifedipine[6], meloxicam[7], and oxazepam[8]. It has attracted much attention because of its negligible toxicity, high aqueous solubility, good flow and compression properties, low hygroscopicity, and physiological acceptance[4]. Although having a relatively high melting point (166–168°C), mannitol has been investigated in the hot melt procedure as a carrier for improving drug solubilization[7].

To date, most of the pharmaceutical researches with sugars have focused on the use of the melting method. However, lately, mannitol has been designated as unsuitable for the preparation of solid dispersions because of

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its polymorphic transition that may occur after melting[9]. In addition, the sugar solubility in most organic solvents is poor, making it difficult to prepare coevaporates. To overcome this problem, lyophilization monophase solution technique was employed by Van Drooge et al.[10] using tertiary butyl alcohol (TBA)–water mixture. They used sugars, namely trehalose, sucrose, and inulin as hydrophilic carriers for the preparation of LDs of lipophilic drugs, namely diazepam, Δ9-tetrahydrocannabinol, and cyclosporine A. Lyophilization monophase solution technique was also used to enhance the dissolution rate of the poorly soluble drugs: budesonide, salmeterol, ketoprofen, nitrendipine, and FLT by complexation with cyclodextrins[3,11].

Arakawa et al.[12] have examined the effects of the amino acid arginine on the solubility of a highly insoluble protein, gluten, and two organic compounds, octyl-gallate and coumarin. Arginine significantly increased the solubility of these molecules. In another study[13], arginine showed a synergistic effect when used in combination with hydroxypropyl-β-cyclodextrin in improving the solubility of naproxen, which suggests that arginine may be useful to enhance the solubility of poorly soluble drug substances.

Until now, no reports about FLT dispersions with sugars or amino acids are available. Therefore, the aim of this work was to improve the FLT dissolution rate using polyols (mannitol and trehalose) and amino acids (arginine and glycine). This was achieved by formulating LDs of FLT with these carriers using lyophilization monophase solution technique. A physicochemical study of the systems under study using thermal analysis, X-ray diffraction, and microscopical methods was conducted. In addition, the particle size, surface area, and porosity of the prepared dispersions were evaluated. Such analysis was done to elucidate the factors responsible for the improvement of the in vitro dissolution of FLT from the lyophilized formulations.

Materials and methods

Materials

FLT was kindly donated by Archimica (Origgio, Italy). Granular mannitol, MANNOGEM™ 2080 was from SPI Polyols (New Castle, England). D-(+)-trehalose dihydrate and TBA were purchased from Sigma-Aldrich (St. Louis, MO, USA), L-arginine from Qualikems (Vadodara, India) and Glycine and Glycocoll from Prolabo (Milano, Italy). All other chemicals were of analytical grade and used without further purification.

HPLC assay for flutamide

A reverse-phase HPLC method was used for quantifying FLT[14]. HPLC analysis was carried out with a Perkin Elmer series 200 chromatograph (Perkin Elmer, Boston, MA, USA) using a Spheri-5, RP-18, 220 × 4.6 mm, 5 μm, column (Perkin Elmer), 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 minutes and the retention time was 3.5 minutes. Calibration curves (peak area versus drug concentration) were linear (r^2 > 0.999) over the FLT concentration range of 0.6–60 μg/mL.

Phase solubility study

Aqueous solubility of FLT in the presence of mannitol, trehalose, arginine, or glycine was carried out according to the method described by Higuchi and Connors[15]. An excess amount of FLT was added to 10 mL of aqueous solutions containing increasing concentrations of the hydrophilic carrier (0, 1, 5, 10, 15, and 20%, w/v) in screw-capped vials. The suspensions were shaken in a thermostatically controlled water bath (GFL, type 1083, Gmbh & Co., Burgwedel, Germany) at 37 ± 0.5°C for 24 hours. 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. The solubilization efficiency of carriers was calculated as the ratio of FLT aqueous solubility at 5% (w/v) carrier concentration and FLT intrinsic solubility in pure water.

Preparation of FLT lyophilized dispersions

FLT–polyol and FLT–amino acid LDs in different mass ratios were prepared by dissolving the calculated amount of hydrophilic carrier in 5 mL water and mixing with FLT/TBA solution (300 mg/5 mL) in 50 mL vials. Immediately after mixing, the vials were frozen at –80°C for 4 hours followed by placing them in a Cryodos-50 lyophilizer (Telstar Cryodos, Terrassa 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 carrier in a mortar and stored at room temperature in hermetically sealed bottles until use.

Characterization of FLT lyophilized dispersions

Residual TBA determination

Based on 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. It is reported that during the secondary drying not all TBA will evaporate from the freeze-concentrated fraction. Therefore, the controlling of residual TBA was needed though TBA is a low toxic organic solvent and has little detriment to human body[16].

The amount of residual TBA in LDs was determined using AutoSystem XL gas chromatograph 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). A known weight of the lyophilized sample was immediately dispersed in 2 mL distilled water and gas chromatograph quantificated the concentration of TBA in 1 μL of the vapor. The injection temperature was 200°C at a rate of 10°C/min. 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.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms of pure materials, PMs, and LDs were recorded by DSC 6 differential scanning calorimeter (Perkin Elmer). 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:

$$\%\ \text{Crystallinity} = 100 \times \frac{\Delta H_s}{\Delta H_c \times C}, \tag{1}$$

where ΔH_s and ΔH_c 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[17].

Powder X-ray diffractometry

The X-ray diffractograms (XRD) of pure materials and LDs were carried out using XRD-7000 X-ray diffractometer (Shimadzu, Kyoto, Japan) where Cu-Kα_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.

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) spectra of pure materials, PMs, and LDs were recorded using a Spectrum RXI FT-IR spectrophotometer (Perkin Elmer) 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.

Scanning electron microscopy

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

Particle size analysis

The particle size of pure FLT and LDs was determined using model 1064 liquid laser diffraction particle size analyzer (Cilas, Orleans, 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.

Surface area and porosity analysis

Specific surface area and porosity of pure FLT and LDs were measured using the NOVA 1000 series surface area analyzer (Quantachrome, FL, USA). A known weight of powder was added to a 12 mm Quantachrome bulb sample cell and degassed for 3 hours prior to analysis. A five-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 theory of surface area[18].

Flow properties

Flow properties of pure FLT and LDs were evaluated by determining the angle of repose and Carr's compressibility index (CI). Static angle of repose was measured according to the fixed funnel and free-standing cone method[19]. The samples were carefully poured through the funnel until the apex of the conical pile formed just reaches the tip of the funnel. The circumference of the pile formed was drawn, the mean diameter (2R) was determined and the tangent of the angle of repose was given by the following equation:

$$\tan\theta = \frac{H}{R}, \tag{2}$$

where θ is the repose angle and $H = 2$ cm.

Carr's CI of the samples was determined using the tapping method according to the following equation:

$$\%\ \text{CI} = 1 - \left(\frac{V}{V_0}\right) \times 100, \tag{3}$$

where V and V_0 are the volumes of the sample after and before the standard tapping, respectively. The tapping was carried out in a 10 mL measuring cylinder after observing the initial volume of the powder. The tapping was continued on a hard surface at a rate of 100 taps/min until no further change in volume was noted.

In vitro dissolution study

FLT dissolution behavior was evaluated using the USP XXIV dissolution rate apparatus II (Pharmatest, Hainburg, Germany) at a stirring rate of 100 ± 2 rpm. Powder samples containing 60 mg of pure FLT or its equivalent amount of LDs or PMs were placed in 900 mL of dissolution fluid (0.1 N HCl, pH 1.2) at 37 ± 0.5°C for 2 hours. At predetermined time intervals, 5 mL 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 using HPLC at 304 nm. The percentage cumulative amount of drug dissolved was plotted against time.

Results and discussion

Phase solubility study

The solubility of FLT in water at 37°C was found to be 21 µg/mL. The effect of the addition of polyols (mannitol and trehalose) and amino acids (arginine and glycine) on the aqueous solubility of FLT was studied. The solubility profiles of FLT in the various aqueous concentrations of these carriers are shown in Figure 1. Arginine and glycine showed A_L-type phase solubility diagrams where the aqueous solubility of the drug increased linearly as a function of amino acid concentration, with the solubilizing efficiency of arginine and glycine at 5% (w/v) being 1.69 and 1.55, respectively[15]. Crowley and Golovin[20] reported that arginine has shown binding to aromatic groups through π electron–cation interaction. As FLT contains aromatic ring, arginine may bind to this compound through this mechanism and hence increase its solubility. On the other hand, a type of phase solubility diagram other than A_L-type was obtained using polyols where a nearly linear correlation was obtained below 5% (w/v) of polyol concentration with a solubilizing efficiency of 1.43 and 1.32 for mannitol and trehalose, respectively. Further increase in the polyol concentration beyond 5% (w/v) led to a decrease in drug solubility.

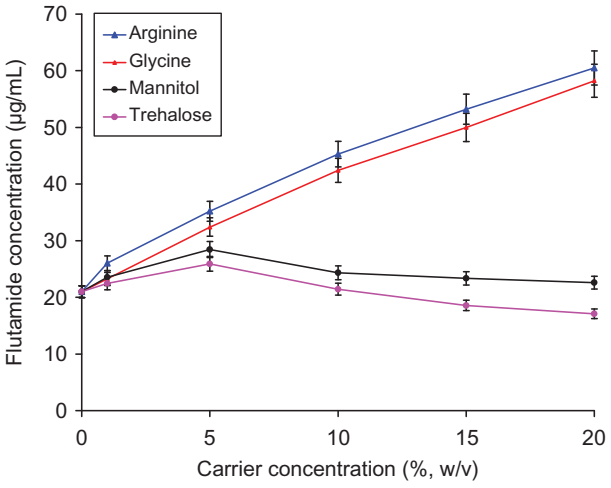

Figure 1. Phase solubility diagram of flutamide in aqueous solutions of mannitol, trehalose, arginine, and glycine at 37°C.

Characterization of FLT lyophilized dispersions

Residual solvent determination

Although not listed in the ICH guidelines for residual solvents, 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 of TBA. Therefore, the low level of TBA in the lyophilized cakes should not be harmful to both animals and humans[21].

In this study, gas chromatography confirmed that there are only 0.015% and 0.020% (w/w) residuals of TBA in the 1:1 FLT–mannitol and FLT–trehalose LDs, respectively. For amino acids, similar results were obtained with 0.007% and 0.023% (w/w) residual TBA were found in the 1 : 1 FLT–arginine and FLT–glycine LDs, respectively, which was much lower than the toxic level. This low level of TBA in the LDs 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[16,21].

Differential scanning calorimetry

Figures 2 and 3; demonstrated the thermograms of FLT–mannitol and FLT–trehalose systems, respectively. The

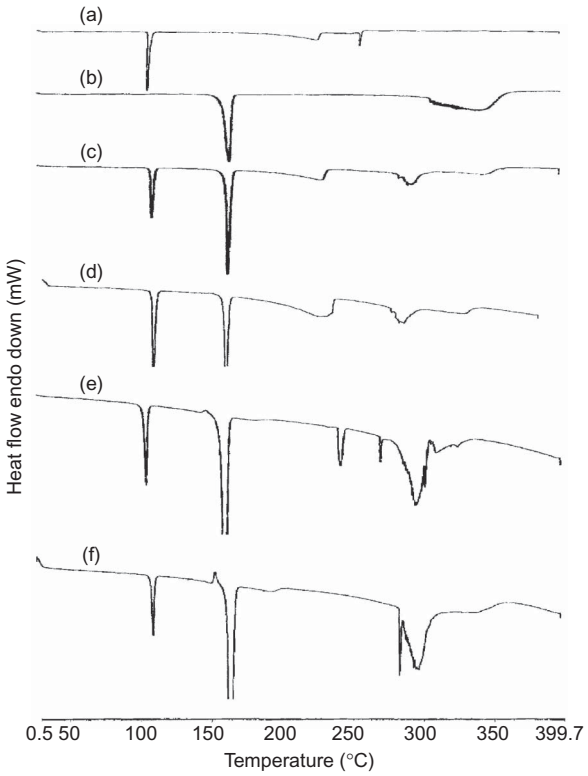

Figure 2. DSC thermograms of FLT–mannitol systems: pure FLT (a), mannitol (b), FLT–mannitol 1 : 1 PM (c), FLT–mannitol 1 : 1 LD (d), FLT–mannitol 1 : 3 LD (e), FLT–mannitol 1 : 5 LD (f).

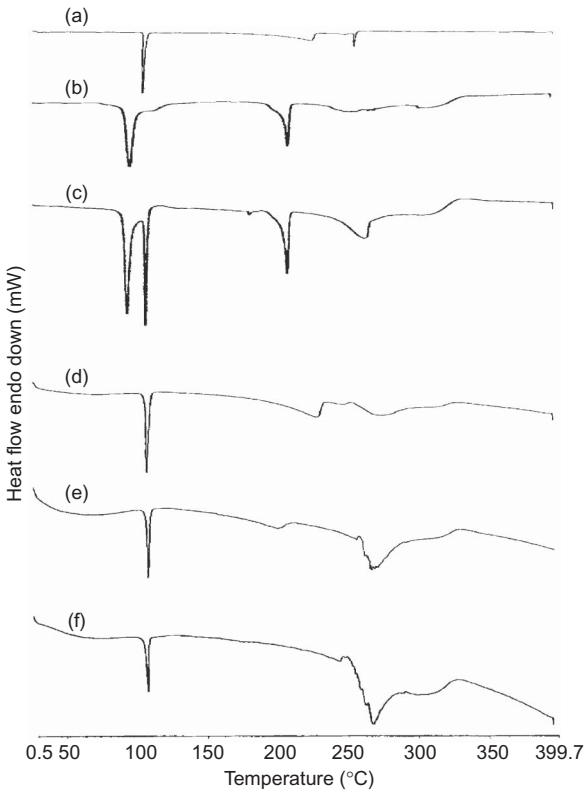

Figure 3. DSC thermograms of FLT–trehalose systems: pure FLT (a), trehalose (b), FLT–trehalose 1 : 1 PM (c), FLT-trehalose 1 : 1 LD (d), FLT–trehalose 1 : 3 LD (e), FLT–trehalose 1 : 5 LD (f).

thermograms of 1:1 FLT–polyol PMs showed two endothermic peaks, corresponding to the melting of both FLT (about 112°C) and polyols (165°C and 205°C for mannitol and trehalose, respectively)[3,7,22]. These melting peaks indicated the crystalline nature of both drug and carriers. In addition, the presence of the peaks separated each at its position indicated the absence of any solid state interaction between them. The DSC thermograms of FLT–mannitol LDs in different ratios ranging from 1:1 to 1:5, were still exhibiting the two peaks corresponding to FLT and mannitol. However, the drug fusion enthalpy was found to decrease with increasing the mannitol content thus resulting in a reduction in the percent drug crystallinity. Trehalose showed a similar behavior although FLT–trehalose LDs showed only the sharp peak of FLT at 112.21°C with disappearance of trehalose peaks indicating its transformation to an amorphous form[22]. The fusion enthalpy (ΔH) of FLT was reduced from 83.86 J/g (pure FLT) to 11.35 and 10.13 J/g in the 1:5 FLT–mannitol and FLT–trehalose LDs, respectively, resulting in a reduction in drug crystallinity to 79.59% and 71.04%, respectively (Table 1).

The DSC thermograms of FLT–arginine and FLT–glycine systems were illustrated in Figures 4 and 5, respectively. The amino acids showed similar results to polyols. In case of 1 : 1 FLT–arginine and FLT–glycine LDs, the characteristic thermal peak of the drug still appeared at its melting temperature but with reduced crystallinity indicating that the crystalline drug existed as dispersion in the carrier. Increasing the amino acid content decreased the drug crystallinity to 73.15% and 62.29% in the 1 : 3 FLT–arginine and FLT–glycine LDs, respectively (Table 1).

Powder X-ray diffractometry

Figure 6a and b showed the X-ray diffractograms of FLT–polyols LDs using mannitol and trehalose. The diffractogram of pure FLT revealed that FLT is a crystalline compound showing a very strong sharp diffraction peak at 2θ of 8.726° whereas other peaks are present at a lower intensity[3]. The characteristic peaks of FLT, mannitol, and trehalose were still present in the LDs in their positions but broader with a lower intensity. These results indicated that the crystallinity of the drug decreased as the mannitol or trehalose content increased in the LD. This is in agreement with the DSC studies confirming that the drug still existed as a crystalline material in the carrier.

Similar observations were noticed in the X-ray diffractograms of FLT–amino acid LDs using arginine and glycine (Figure 6c and d, respectively). The characteristic diffraction peak of the drug in these dispersions was also broadened with its intensity reduced compared to pure drug.

Table 1. Physicochemical properties of FLT and lyophilized dispersions with polyols and amino acids (values are mean ± SD, $n = 3$).

Formula	Particle size (μm)	Specific surface area (m^2/g)	Total pore volume (e^{-3} mL/g)	Angle of repose (θ)	Compressibility index (% CI)	% Drug crystallinity
FLT	2.96 ± 0.03	233.42 ± 5.06	339.64 ± 4.71	57.23 ± 2.24	65.45 ± 3.03	100
FLT-Man 1 : 1	1.81 ± 0.05	293.93 ± 7.60	503.72 ± 10.4	53.06 ± 2.09	47.43 ± 3.45	99.26
FLT-Man 1 : 3	1.18 ± 0.06	343.56 ± 8.43	533.07 ± 7.48	44.42 ± 7.46	42.86 ± 4.72	93.29
FLT-Man 1 : 5	0.75 ± 0.08	370.21 ± 7.23	589.62 ± 9.89	41.63 ± 7.24	38.89 ± 4.22	79.59
FLT-Tre 1 : 1	1.00 ± 0.09	322.51 ± 5.923	669.63 ± 5.83	48.65 ± 6.26	62.45 ± 8.35	98.35
FLT-Tre 1 : 3	0.72 ± 0.08	365.11 ± 5.42	703.65 ± 6.43	44.71 ± 2.33	60.00 ± 4.67	88.14
FLT-Tre 1 : 5	0.61 ± 0.04	426.37 ± 6.73	754.33 ± 2.78	40.39 ± 5.28	56.44 ± 3.45	71.04
FLT-Arg 1 : 1	1.00 ± 0.03	300.22 ± 9.03	447.69 ± 11.6	55.03 ± 5.12	50.00 ± 5.22	96.22
FLT-Arg 1 : 3	0.91 ± 0.04	345.55 ± 8.53	523.36 ± 7.49	49.72 ± 6.29	40.00 ± 5.83	73.15
FLT-Gly 1 : 1	1.00 ± 0.10	405.45 ± 6.34	589.44 ± 5.37	49.96 ± 6.35	54.55 ± 6.35	69.44
FLT-Gly 1 : 3	0.81 ± 0.07	465.37 ± 4.77	643.57 ± 8.77	46.12 ± 2.93	45.45 ± 2.10	62.29

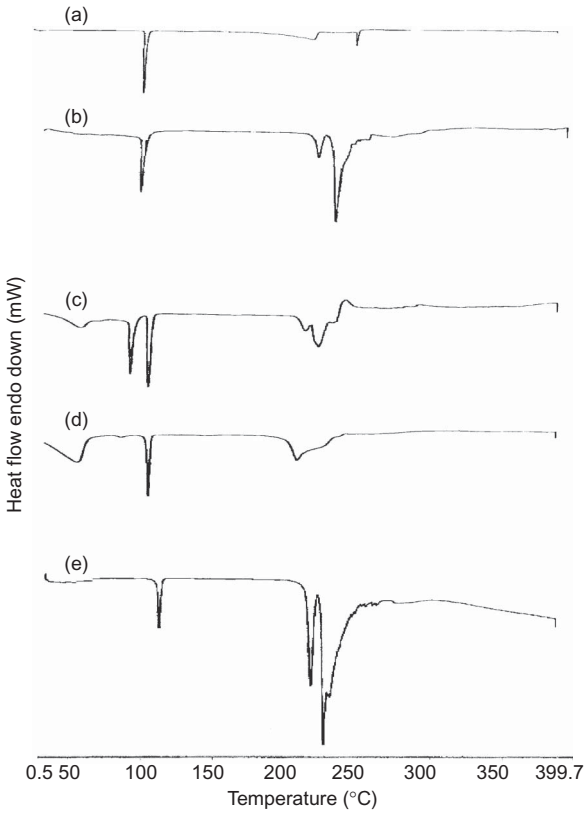

Figure 4. DSC thermograms of FLT–arginine systems: pure FLT (a), arginine (b), FLT–arginine 1 : 1 PM (c), FLT–arginine 1 : 1 LD (d), FLT–arginine 1 : 3 LD (e).

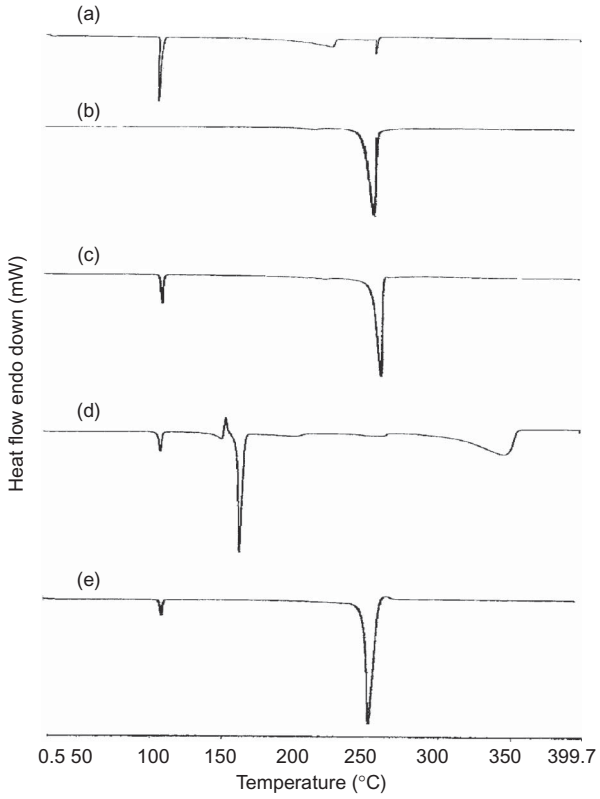

Figure 5. DSC thermograms of FLT–glycine systems: pure FLT (a), glycine (b), FLT–glycine 1 : 1 PM (c), FLT–glycine 1 : 1 LD (d), FLT–glycine 1 : 3 LD (e).

Fourier transform infrared spectroscopy

The IR transmission spectra of FLT LDs and PM with mannitol are demonstrated in Figure 7. In the PMs, all characteristic peaks of FLT are present in their original positions denoting the absence of drug–carrier interaction. The characteristic peak of FLT at 3360 cm^{-1} corresponding to its amino group was also detected in the FLT–carrier LDs but with its intensity decreases as the carrier content increases. These findings are in agreement with the results obtained by DSC and XRD studies. Similar IR transmission spectra of FLT LDs and PMs with trehalose, arginine, and glycine were obtained (data not shown).

Scanning electron microscopy

Figure 8 illustrates the images of pure FLT and its 1 : 1 LDs with polyols and amino acids under study. Pure FLT image showed crystalline drug of plate-like crystals. The presence of small drug crystals uniformly and finely dispersed or adhered to the surface of carrier particles were clearly detectable in the FLT LDs with all carriers thus confirming the presence of free crystalline drug. Similar findings were obtained with Arias et al.[4] where the triamterene–mannitol solid dispersion demonstrated crystalline particles of variable dimensions. In contrast, Mura et al.[13] showed that naproxen and arginine crystals were not detectable in the micrograph of their coground system because of the formation of amorphous aggregates.

Particle size analysis

Literature lacks sufficient data about the influence of polyols or amino acids on the particle size of drug–solid dispersions. Therefore, our study gave a special emphasis to the particle size of FLT LDs and consequently on their surface area and porosity. The particle size measurements of pure FLT and its LDs with polyols and amino acids are listed in Table 1. The average particle size of FLT crystals was 2.96 μm. It was observed that lyophilization technique reduced the particle size of the lyophilized drug dispersions. In addition, the decrease in the particle size might be a factor of the carrier nature and ratio. In this study, the particle size of FLT LDs was in the range of 0.61–1.81 μm corresponding to FLT–trehalose 1:5 and FLT–mannitol 1:1 LDs, respectively. It was also observed that the particle size of the prepared FLT LDs decreased as the carrier content increased.

Surface area and porosity analysis

An important factor that influences the dissolution rate is the available surface area of the active pharmaceutical ingredient. The surface area per unit powder mass was

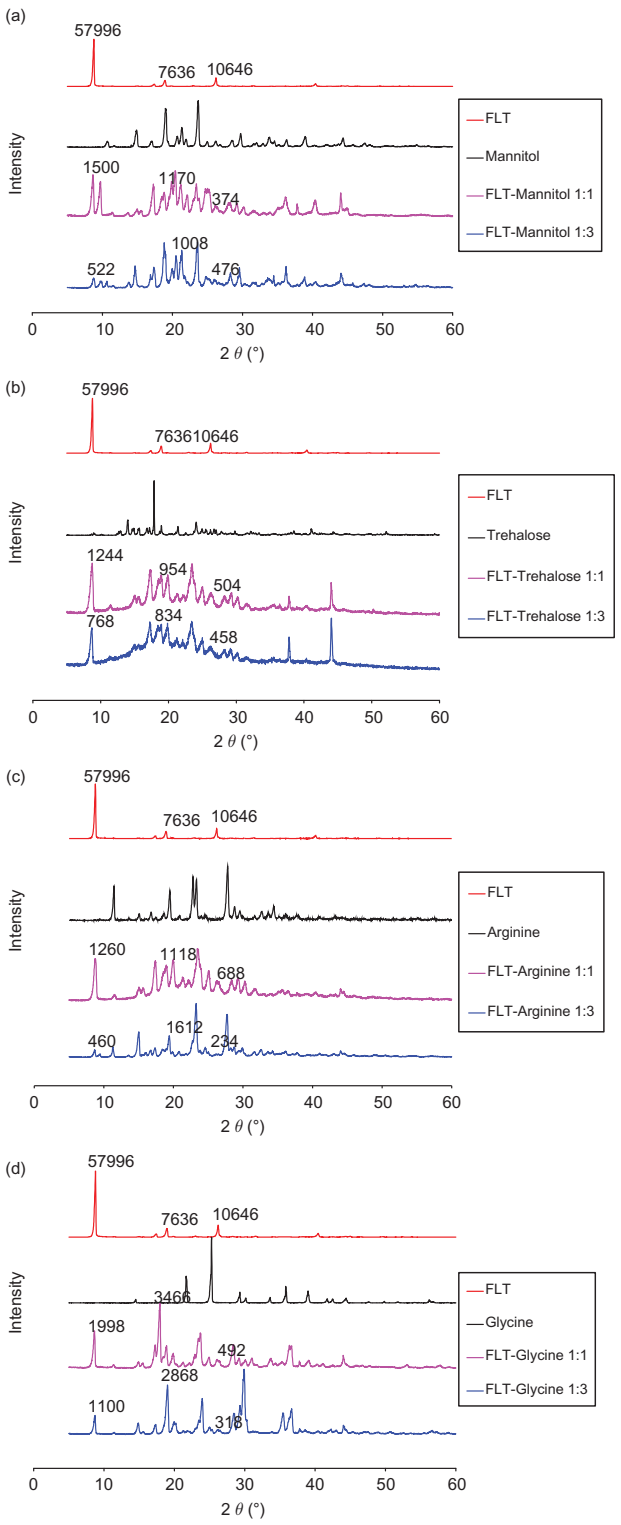

Figure 6. (a) X-ray diffractograms of flutamide–mannitol lyophilized dispersions; (b) flutamide–trehalose lyophilized dispersions; (c) flutamide–arginine lyophilized dispersions; (d) flutamide–glycine lyophilized dispersions.

calculated from the fit of nitrogen adsorption data to the Brunauer–Emmett–Teller equation. The determined specific surface area and total pore volume of pure FLT and its LDs with polyols and amino acids are listed in

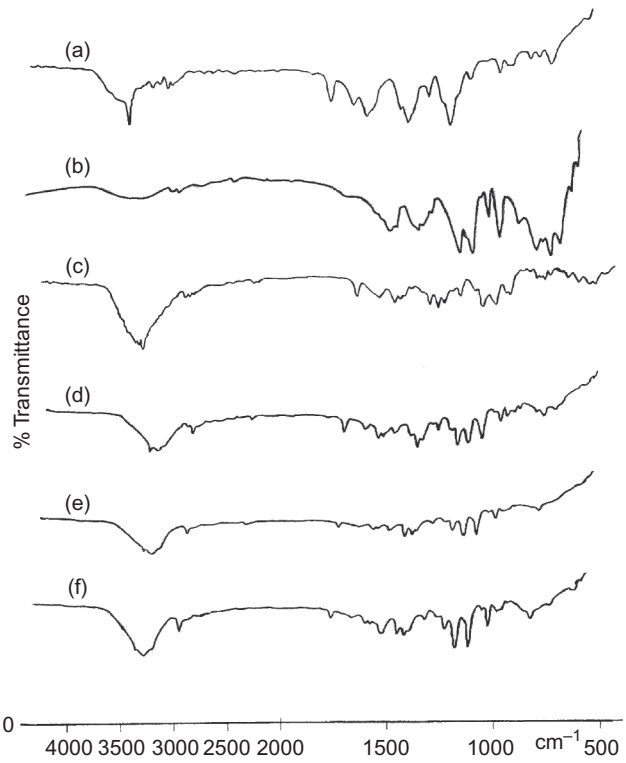

Figure 7. FTIR transmission spectra of pure FLT (a), mannitol (b), FLT–mannitol 1 : 1 PM (c), FLT–mannitol 1 : 1 LD (d), FLT–mannitol 1 : 3 LD (e), FLT–mannitol 1 : 5 LD (f).

Table 1. 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. Polyols and amino acids showed an increase in the drug specific surface area to 293.93–465.37 m^2/g corresponding to FLT–mannitol 1:1 and FLT–glycine 1:3 LDs, respectively. The total pore volume of FLT LDs was also increased to 447.69–754.33 e^{-3} mL/g for FLT–arginine 1:1 and FLT–trehalose 1:5 LDs, respectively. Such a high surface area indicated that the lyophilized powder particles were highly porous. Their highly porous structures may be attributed to the channels created as the solvent was removed during the sublimation process in the lyophilization cycle. These results were in agreement with the work done by Williams III et al.[23]

Flow properties

The flow properties of pure drug and its LDs with sugars and amino acids under study were evaluated by measuring the angle of repose and Carr's CI (Table 1). The drug showed a poor flow property as indicated by the high value of angle of repose (57.23°) and percent CI (65.45%). The technique and carriers used improved the flow property of the drug but to a limited extent. The angle of repose of FLT LDs ranged from 40.39° to 55.03° therefore most of LDs powder has poor flowability except for FLT–Tre 1:5 which may have fair flowability. However, a drug load up to 25% (w/w) (drug : carrier, 1:3) incorporated in the carrier can improve the flow properties of drug but

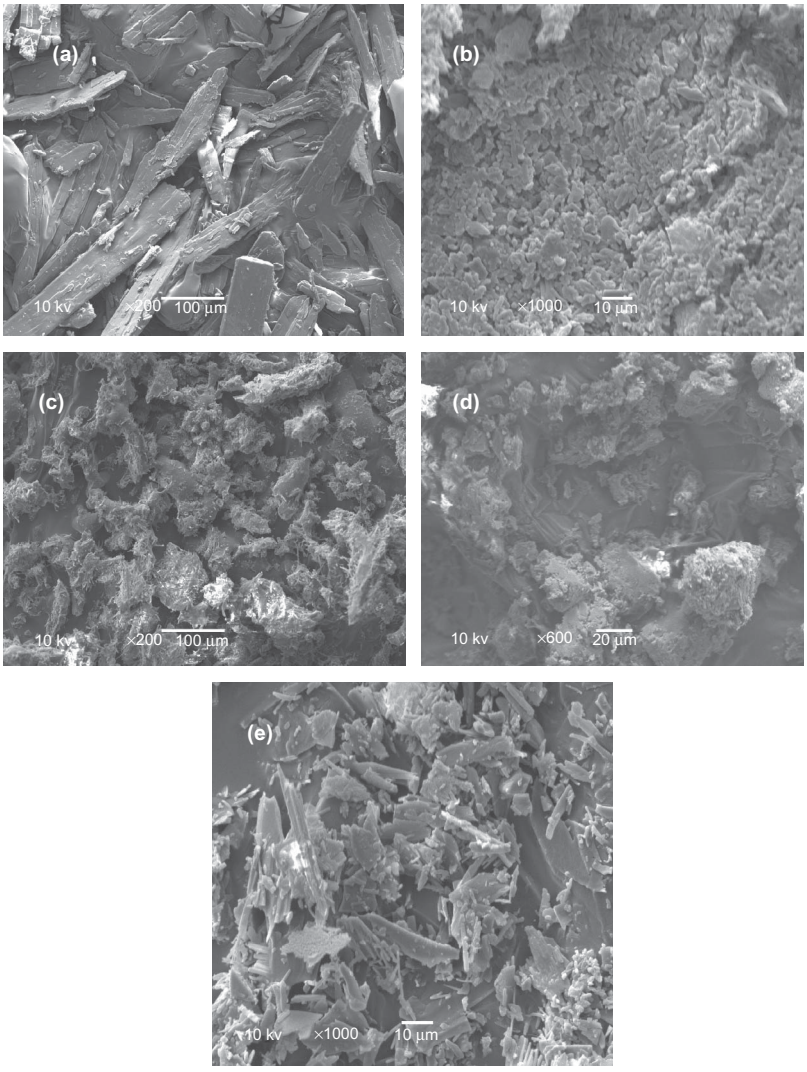

Figure 8. Scanning electron micrographs of selected 1 : 1 FLT–carrier LDs: pure FLT (a), FLT–mannitol (b), FLT–trehalose (c), FLT–arginine (d), FLT–glycine (e).

to a limited extent. The relatively high value of angle of repose indicated the roughness of the particle surface that may hinder the flow of the drug particles. As the roughness of the surface increased, the points of contact and so the friction between particles increased that resisted the flow of the particles relative to each other[24].

Concerning the percent CI, the FLT LDs exhibited CI value between 38.89% and 62.45% corresponding to FLT–mannitol 1:5 and FLT–trehalose 1:1 LDs, respectively. The poor flow and high CI of the LDs could be also attributed to the nature of the LDs which are usually soft and tacky[24].

In vitro dissolution study

For all FLT LDs with polyols and amino acids under study and their corresponding PMs; four parameters, dissolution efficiency calculated after 60 minutes ($\%DE_{60}$), percentage of dissolved drug after 30 minutes (PD30), relative dissolution rate after 5 minutes (RDR**5**), and time required to dissolve 25% of drug (T25%) were measured (Table 2). The dissolution rate of pure FLT was very slow that after 120 minutes about 19% of the drug was dissolved. This poor dissolution behavior of the drug could be explained on the basis of its low aqueous solubility, poor wettability, and/or agglomeration[3].

According to Table 2, FLT dissolution was slightly enhanced when physically mixed with the polyols with respect to FLT alone. This is in good correlation with the study of Ginés et al.[25] where mannitol increased the solubility of triamterene from their PMs and this solubilizing effect increased the dissolution rate of triamterene. Mannitol was found to strengthen intermolecular hydrogen bonding in water, making the solvent more hydrophobic and thus favoring the dissolution of the hydrophobic drug.

The dissolution results revealed that the LD formation resulted in a marked increase of FLT dissolution comparing to PMs. Formation of 1:1 FLT–mannitol and

Table 2. Dissolution parameters of FLT, physical mixtures, and lyophilized dispersions with polyols and amino acids 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	10.8 ± 0.42	1	13.45 ± 0.50	120 ± 2.11
FLT-Man 1 : 1 PM	16.41 ± 3.95	4.26 ± 0.06	20.77 ± 2.45	69 ± 2.20
FLT-Man 1 : 1 LD	26.12 ± 4.17	4.63 ± 0.37	30.34 ± 1.45	18 ± 1.06
FLT-Man 1 : 3 LD	34.71 ± 2.31	10.74 ± 0.83	38.36 ± 1.69	7 ± 0.15
FLT-Man 1 : 5 LD	42.61 ± 2.63	19.05 ± 1.20	46.35 ± 3.56	3 ± 0.04
FLT-Tre 1 : 1 PM	16.33 ± 3.27	3.29 ± 0.08	17.88 ± 4.58	100 ± 2.16
FLT-Tre 1 : 1 LD	19.99 ± 3.99	6.39 ± 2.84	21.60 ± 1.22	45 ± 3.73
FLT-Tre 1 : 3 LD	23.78 ± 2.09	6.08 ± 0.05	25.86 ± 2.57	30 ± 0.84
FLT-Tre 1 : 5 LD	33.12 ± 1.41	10.37 ± 1.04	36.43 ± 2.45	10 ± 0.38
FLT-Arg 1 : 1 PM	16.71 ± 2.40	3.92 ± 0.93	18.15 ± 0.74	100 ± 3.74
FLT-Arg 1 : 1 LD	28.84 ± 0.58	9.58 ± 0.45	32.39 ± 2.55	18 ± 0.46
FLT-Arg 1 : 3 LD	36.84 ± 1.52	11.88 ± 0.54	38.57 ± 2.05	6 ± 0.02
FLT-Gly 1 : 1 PM	18.21 ± 0.53	3.70 ± 0.04	20.53 ± 0.94	90 ± 3.06
FLT-Gly 1 : 1 LD	32.39 ± 0.39	6.03 ± 0.12	36.31 ± 2.40	9 ± 0.83
FLT-Gly 1 : 3 LD	40.08 ± 3.85	9.92 ± 0.13	46.78 ± 4.33	9 ± 1.73

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

FLT–trehalose LDs increased the $\%DE_{60}$ value from 10.83% (pure FLT) to 26.12% and 19.99%, respectively. After 30 minutes, there were almost 30.34% and 21.60% of drug dissolved from both types of dispersions, respectively, whereas the FLT alone resulted in only 13.45% dissolution. It was also concluded that the dissolution rate of FLT increased upon increasing the polyol content. Increasing the carrier content in the 1:3 FLT–mannitol and FLT–trehalose LDs increased the FLT $\%DE_{60}$ to 34.71% and 23.12%, respectively.

Similar results were obtained using amino acids (arginine and glycine) but with a relatively higher dissolution enhancing effect. The $\%DE_{60}$ of FLT from its 1:1 LDs with arginine and glycine was 28.84% and 32.39%, respectively. Increasing the amino acid content increased the drug $\%DE_{60}$ to 36.84% and 40.08%, in the 1:3 FLT–arginine and FLT–glycine LDs, respectively.

Typical mechanisms for improvement of dissolution characteristics of drugs via solid dispersions are particle size reduction, the absence of crystal structure, and improved wettability[26]. In a previous study, we attributed the enhanced FLT dissolution mainly to the formation of soluble inclusion complexes of the drug with CDs (βCD and HPβCD) and the high energetic amorphous state following complexation[3]. However, in the current study, the information supplied by DSC and XRD indicates that there was no amorphous FLT present with polyols or amino acids (Figures 2 and 3), and SEM micrographs confirmed these observations (Figure 8). Higher dissolution rate of FLT in these LDs was therefore attributed to partial loss of crystallinity and the reduced particle size. This explanation is strengthened by the results of surface area and total pore volume (Table 1). Additionally, the improved wetting of the crystal surface mainly because of attached carrier particles, provoked the solubilizing effect. The carrier attracts the dissolution medium and increases its amount in the immediate vicinity of the FLT surface. Furthermore, the arrangement of carrier physically separates drug particles, preventing their aggregation after introduction of the solid-dispersed system to the dissolution medium[27].

Conclusions

Our study demonstrated that lyophilization monophase solution technique was suitable for obtaining solid homogenous dispersions of FLT with polyols and amino acids with a low residual TBA. The improvement in dissolution rate of FLT was not because of the amorphization of FLT as indicated by DSC and XRD studies and confirmed by SEM results. However, other parameters such as reduced crystallinity, increased solubility, reduced particle size, and increased surface area and porosity were found to contribute to the enhanced dissolution of FLT. The IR results suggested that there was no chemical interaction between FLT and the carriers. Also the carriers could improve the poor flow properties of FLT but to a limited extent. These systems could be useful for formulating fast-dissolving drug solid dosage forms able to assure rapid onset of action and improved bioavailability.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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